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Intensification of Biomass Fractionation Processes Using Solid Catalysts

Thesis to obtain the Master of Science Degree in

Chemical and Biochemical Engineering

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Abstract

The development of selective and effective hemicellulose recovery and upgrade processes is one of the current major hurdles preventing the full onset of economic and environmentally sustainable biorefineries.

In this work, four ion exchange resins (IERs) were characterized and evaluated as alternative catalysts, in order to explore the advantages of the acid-catalyzed processes for the i) direct hydrolysis of raw biomass and ii) the hydrolysis of oligosaccharides, both into monomeric hemicellulose-derived sugars.

The hemicellulose hydrolysis using IERs was compared to the use of dilute sulfuric acid for diverse model feedstocks. For H_2SO_4 processes, xylan conversion into xylose reached 83.9% for Eucalyptus residues (ER), and 98.6% for Miscanthus. When using IERs, a superior or equivalent performance, namely, xylose yields of 93.7% (ER), and 91.3% (Miscanthus) were achieved when using Amberlyst 15. This was further validated for Wheat Straw (WS) that presented a complete hydrolysis of xylan into xylose, showing that IERs usage is an effective alternative for biomass deconstruction.

In the two-step process, autohydrolysis and organosolv were compared as means to produced Xylo-oligosaccharides (XOS) both from ER and WS. Autohydrolysis presented higher XOS yields for both materials and these could be effectively hydrolyzed by IERs, namely Amberlite IR120 and specially Amberlyst 15, enabling to reach efficiencies superior to the H_2SO_4 catalyzed process. Catalyst reutilization is possible, and typically higher in autohydrolysis when compared to organosolv derived streams. This process was demonstrated to be able to be further intensified by the use of a continuous system, using raffinose as model oligosaccharide, for which a 50% hydrolysis was achieved for a low residence time (5 min) at 140ºC.

Finally, the performance of the IERs is discussed based on their structures and properties and the impact of the amount of ash present in the feedstock.

Keywords

Acid hydrolysis
Hemicellulose
Ion exchange resins
Lignocellulosic biomass
Solid acids

Resumo

O desenvolvimento de processos seletivos e eficientes de recuperação de hemiceluloses é um dos principais obstáculos ao aparecimento de biorrefinarias sustentáveis a nível económico e ambiental.

Neste trabalho, foram caracterizadas e avaliadas quatro resinas de troca iónica (IERs) como catalisadores ácidos alternativos, com o objetivo de explorar as vantagens dos processos ácidos para a i) hidrólise direta de biomassa e ii) hidrólise de oligossacáridos, ambas em monómeros de açúcares derivados de hemicelulose.

A hidrólise da hemicelulose usando IERs foi comparada ao uso de ácido sulfúrico diluído para diversas matérias-primas. Nos processos catalisados por H_2SO_4, a conversão de xilano em xilose atingiu 83,9% para resíduos de eucalipto (ER) e 98,6%, para Miscanthus. As IERs, apresentaram um desempenho superior ou equivalente, com rendimentos de xilose de 93,7% (ER) e 91,3% (Miscanthus) usando Amberlyst 15. Isto foi posteriormente validado para a palha de trigo (WS) que apresentou hidrólise completa de xilano em xilose, demonstrando que as IERs são uma alternativa eficaz para desconstrução da biomassa.

No processo de duas etapas, a auto-hidrólise e o organosolv foram comparados como processos de produção de Xilo-oligossacáridos (XOS) para ER e WS. A autohidrólise apresentou maiores rendimentos em XOS, para ambos os materiais. O XOS obtidos puderam ser eficazmente hidrolisados por IERs, nomeadamente Amberlite IR120 e principalmente Amberlyst 15, permitindo eficiências superiores ao processo catalisado por H_2SO_4. A reutilização do catalisador é possível, e normalmente é maior nos licores de auto-hidrólise em comparação com os licores organosolv. Este processo demonstrou poder ser ainda intensificado pelo uso de um sistema contínuo, utilizando rafinose como oligossacárido modelo, onde foi obtida uma hidrólise de 50% para um tempo de residência baixo (5 min) a 140ºC.

Por fim, o desempenho das IERs é discutido com base nas suas estruturas e propriedades e no impacto da quantidade de cinza presente na matéria-prima.

Palavras-chave

Ácidos sólidos;
Biomassa lenhocelulósica
Hemicelulose
Hidrólise ácida
Resina de troca iónica

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Notation

AFEX	Ammonia fiber explosion
AOS	Arabino-oligosaccharides
ASE	Accelerated Solvent Extractor
BAS	Brönsted acid sites
C5	Pentoses
C6	Hexoses
CAPEX	Capital expenditure
DAD	Diode array detector
DP	Degree of polymerization
DVB	Divinylbenzene
ER	Eucalyptus residues
GOS	Gluco-oligosaccharides
HMF	Hydroximethylfurfural/ 5-(hydroxymethyl)furfural
HPLC	High-pressure liquid chromatography
IER	Ion Exchange resin
IL	Ionic liquid
Ka	Acid dissociation constant
LHW	Liquid hot water
Log R_o	Severity factor
LSR	Liquid-to-solid ratio
mCS	Modified combined severity factor
NREL	National Renewable Energy Laboratory (USA)
OPEX	Operational Expenditure
pKa	Logarithmic acid dissociation constant
PTFE	Polytetrafluoroethylene
R^2	Coefficient of correlation squared
RID	Refractive index detector
UV	Ultraviolet radiation
WAC	Weakly acidic cation
WS	Wheat straw
wt%	Mass fraction of a component, in percentage
XOS	Xylo-oligosaccharides

Framework

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1 Introduction

1.1 Context and motivation

Due to the shortage of the fossil fuels, the most used resource in the chemical industry for years, some environmental concerns related with the greenhouse effects of the gases originated in those processes, global warming, and increasing price and unexpected fluctuations, there is the need of new sources of energy, chemicals, and materials. Furthermore, the global population keeps growing to unprecedented numbers, and with it the demand for energy and chemical supplies (1, 2).

Biomass is the renewable source with the largest potential for energy, biofuels and chemicals production, and its use is strongly encouraged by the Europe Union (3, 4).

The EU countries are mandated to meet by 2020 a target of 20% renewable resources in the energy supply and 10% renewable resources in energy in the transport sector. The Energy Strategy 2020 (5) of the European Commission calls for increased use of renewable resources in the energy system and the European Council has presented a long-term target for the EU and other industrialized countries of 80 to 95% cuts in greenhouse gas emissions by 2050. A cornerstone in renewable energy projections of the European Union, as mentioned before, is biomass, which is expected to account for 56% of the renewable energy supply in the EU27 by 2020 (6).

The main advantages associated with biomass use, as compared with “fossil resources”, are: i) the decrease of environmental and economic concerns; ii) the increasing security of supply by a multiplicity of sources and possibilities of use; and iii) its great potential when used as a source of raw materials for the production of renewable biofuels, biomaterials and chemical products. This is easily accomplished by the implementation of the biorefinery concept (3). For example, many biofuels can be produced from biomass, such as methanol, ethanol, hydrogen, dimethyl ether, synthetic natural gas, synthetic diesel, bio-oil, and biodiesel, among others (3, 4).

1.2 State of the art

1.2.1 The biorefinery

The biorefinery is an overall concept of an integrated and diversified processing plant where biomass feedstocks are converted into a wide range of valuable products, following the idea of petroleum refineries. Integrated biorefinery is a processing facility that extracts carbohydrates, oils, lignin, and other materials from biomass, converts them into fuels, high-value chemicals, and other materials, with a zero-waste approach (7) (Figure 1).

The International Energy Agency (IEA), in IEA Bioenergy Task 42 "Biorefining in a Circular Economy", characterizes biorefinery as “the sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat)”. Furthermore, IEA recognizes the “important role of biorefineries in the transition towards a sustainable circular economy”, which involves a decline of waste and pollution, the maintenance of products and materials in use, and the regeneration of natural systems.

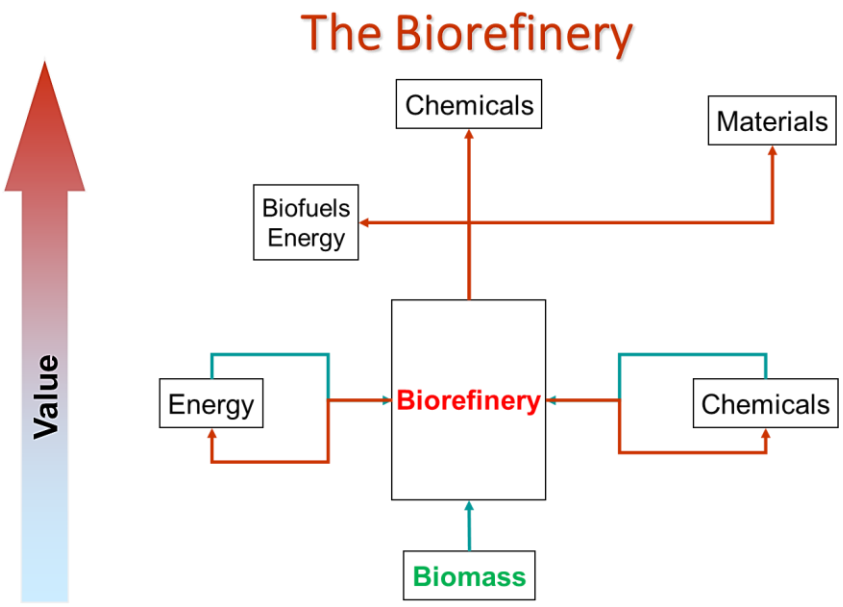

Figure 1-1-The biorefinery concept adapted from (7)

Biorefineries have the potential for the reuse and valorization of waste biomass while facilitating the recycling of carbon after efficient uses and having the option to deliver sustainable power source just as a wide scope of all-around bioproducts, which even incorporate feedstocks for plastics, taking into consideration a total substitution of the petroleum refineries. Biocascading ensures that materials can be kept in the economy for longer, and the final step can deliver renewable energy (8). As shown in Figure 1, the fundamental motivation behind biorefineries in the circular economy is to increase the value of broadly accessible biomass feedstocks. Additionally, the represented recycling of energy and chemical used to convert biomass into high-value products in the biorefinery is the key aspect which decides its sustainable character.

In 2008, the IEA Bioenergy Task 42 developed a biorefinery classification system based on feedstocks, biorefinery platforms, products, and processes (9, 10). The biorefinery feedstocks include grasses, starch crops (wheat and maize), sugar crops (beet and cane), lignocellulosic crops, lignocellulosic residues (stover and straw), oil crops, aquatic biomass (algae and seaweeds) and organic residues (industrial, commercial and post-consumer waste). The IEA classified the processes used in a biorefinery into four groups: 1. Mechanical/Physical, such as pre-treatment, milling, pressing, separation, and distillation, which perform size reduction or separation of feedstock components without affecting the nature of chemical components of the biomass; 2. Biochemical, those processes carried out by enzymes or microorganisms, such as fermentation, anaerobic digestion, etc; 3. Chemical, hydrolysis, synthesis, hydrogenation, oxidation, etc.; and 4. Thermochemical, where feedstocks are subjected to very high temperature and/or pressure, such as gasification, hydrothermal upgrading, and pyrolysis.

The biomass feedstocks can be processed into a variety of biorefinery platforms, which are the key intermediates linking the feedstocks and their respective final product(s). Examples of important platforms in the energy sector are syngas from gasification, biogas from anaerobic digestion, C5 and

C6 sugars from starch, cellulose, and hemicellulose, lignin from lignocellulosic biomass, pyrolysis liquid from pyrolysis, oil from oilseed crops and algae, organic juice from wet biomass, and electricity and heat. These platforms are then further transformed into a variety of products using a thermal, biological or chemical process, or a combination of these processes.

Based on the type of products produced, biorefineries are classified into energy-driven or material-driven biorefinery systems. In an energy-driven biorefinery system, biomass is used mainly to produce biofuels, power, and heat. Whereas, in a material-driven biorefinery system biobased product such as food, feed, chemicals, biomaterials are produced. The process residues, in both systems, can be further utilized to produce energy, thus minimizing waste generation A few examples of this IEA biorefinery classification system are: 1. One-platform C6 sugar biorefinery to produce bioethanol and animal feed from corn crops. 2. One-platform syngas biorefinery for biofuels and chemicals generation from lignocellulosic residues. 3. Two-platform (biogas and organic juice) biorefinery for biomethane, chemicals, biomaterials (fiber products), and fertilizer from grasses. 4. Four-platform (lignin/syngas, C5/C6 sugar) biorefinery for liquid biofuel, bioethanol, and animal feed from lignocellulosic crops such as switchgrass (11).

1.2.2 Lignocellulosic feedstock

Lignocellulosic biomass is the most widely available type of raw material that can be upgraded to various products in a biorefinery, which includes the nature-dry lignocellulosic feedstock such as wood material, straw, corn stover, other agricultural residues (stem, leaves, roots, etc.), energy crops, and municipal lignocellulosic waste. This feedstock's major biopolymers constituents are hemicellulose, cellulose (a polymer of glucose unit), and lignin (polyphenolic compounds). These constituents offer the potential to produce a wide range of value-added products and bioenergy carriers after suitable pre-treatment and processing (12).

1.2.2.1 Cellulose

Cellulose is the main component in most lignocellulosic biomass, representing 35-50% of its mass, and is located predominantly on the secondary wall. It is a polymer made up of β-D-glucopyranose molecules joined by β- (1,4) glycosidic bonds. Each glucose molecule has a 180º rotation concerning neighboring molecules, so the basic repetition unit is a cellobiose molecule (13). This structure allows the establishment of links between the various cellulose chains. The orientation of hydrogen bonds and bonds between polymeric chains make cellulose a highly stable and crystalline polymer, presenting a high degree of polymerization (14).

The cellulose chains are arranged neatly, aggregating in microfibrils, occurring along the chains zones of greater order (crystalline) and which constitute most of the cellulose, and with zones of less order (amorphous). As a result of its crystalline structure and hydrogen bonds, cellulose has a high chemical resistance and is insoluble in most solvents (15, 16). The microfibrils are then encapsulated into larger structures, macrofibrils of 50-250 nm (diameter), in which cellulose is interconnected to hemicellulose via hydrogen bonds, residing within a complex non-crystalline matrix of hemicellulose and lignin (17, 18).

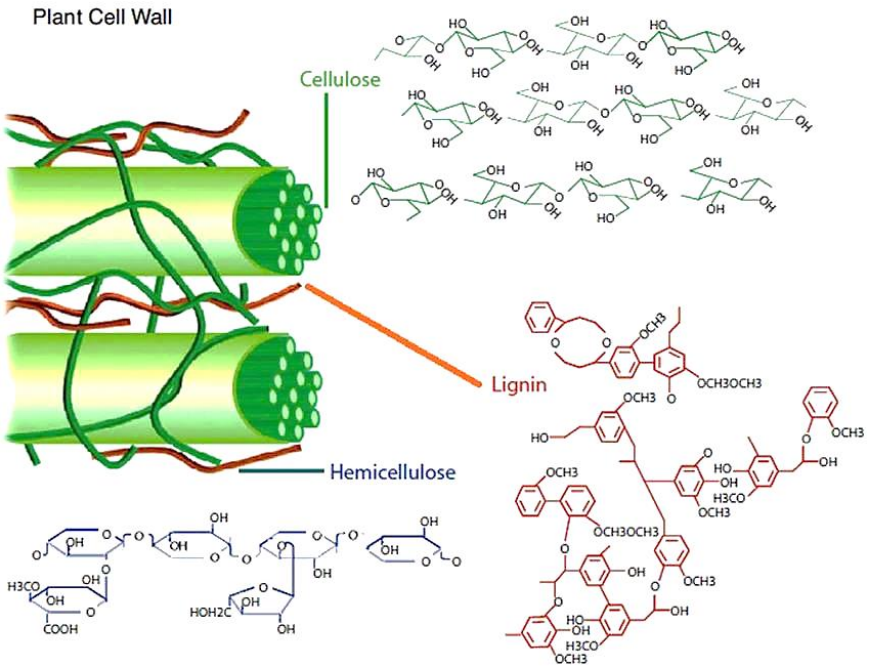

Figure 1-2-Lignocellulosic structure adapted from (19)

1.2.2.2 Hemicellulose

Hemicelluloses are the second most abundant polymer in lignocellulosic biomass (20-50%).

They are heteropolymers made up of various sugars, comprising two large groups: pentoses (β-D-xylose, α-L-arabinose) and hexoses (β-D-mannose, β-D-glucose, α-D-galactose). It also presents small amounts of L-rhamnose, L-fucose, and uronic acids (α-D-glucuronic acids, α-D-4-O-methylglucuronic, α-D-galacturonic). The hydroxyl groups of sugars can be partially replaced by acetyl groups (20).

Among the structural components of the cell wall, hemicelluloses are more susceptible to chemical and thermochemical treatments, a characteristic attributed in large part to their amorphous character and the low degree of polymerization (compared to cellulose), allowing it to be more easily hydrolyzed in acidic media. and soluble in alkaline media, at room temperature. Its solubilization occurs from 180ºC.

Hemicelluloses have differences in structure and composition depending on their biological origin. In hardwoods, the predominant hemicelluloses are xylans (O-acetyl-methylglucuronoxylan) and glucomannans (in lesser quantities). On the other hand, in the softwoods, hemicelluloses are mostly of the galactoglucomannan type (O-acetyl-galactoglucomannan), also containing some xylans (arabino-4-O-methylglucuronoxylan) (21). However, softwoods have a higher proportion of mannose and glucose units compared to the hemicelluloses of hardwoods and agricultural residues. The mannose content can reach 10% in hardwoods and up to 5% in agricultural residues. In these last two groups of materials, in general, about 80% of the hemicellulosic sugars correspond to xylose, so it is frequent to associate the content in hemicelluloses with the content in xylans. The most common xylans are formed by a xylose main chain linked by β-1,4 bonds, where the structural units are replaced by arabinose, glucuronic, methyl-glucuronic, and acetic acid (22).

Hemicelluloses are soluble in alkaline solutions and easily hydrolyzed by acids in their monomeric components, presenting lower chemical and thermal stability than cellulose, probably due to the lack of crystallinity and the lower degree of polymerization. From the hydrolysis of hemicelluloses, hydrolysates are obtained, which may contain hexoses (glucose, mannose, and galactose), pentoses (xylose and arabinose), small amounts of other hexoses (fucose and rhamnose) and even uronic and acetic acids that are linked to some sugars (23).

1.2.2.3 Lignin

Lignin is a heteropolymer complex of high molecular weight and polyphenolic nature, consisting of basic units of phenylpropane, linked by ether and carbon-carbon bonds with different types of bonds. It has a complex three-dimensional structure and difficult microbial degradation (21, 23). Its function in lignocellulosic biomass is to reinforce and waterproof the cell wall, having a fundamental role in the mechanical support, in the conduction of solutes and the protection against external agents in the upper plants (16, 21).

Lignin is an amorphous polymer, built from three units of phenyl-propane, p-coumaryl alcohol, coniferyl alcohol, and synaphyl alcohol, linked by C-O-C (ether) and C-C bonds (Figure 1-3).

These monomeric structures contain the same phenyl-propane backbone, differing only in the degree of substitution of the carbon atom in the aromatic ring that is designated, according to the substituents present such as guaiacyl (G), syringyl (S) and hydroxyphenyl (H) derivatives of coniferyl alcohol, synaphyl alcohol, and p-coumaryl alcohol, respectively (21, 23). The percentage of the three precursors varies according to the origin of the lignocellulosic biomass. Lignin composition will be different not only between species, but also between different tissues of an individual plant variation may occur. Lignins from softwoods are usually referred to as type G lignins because their structural elements result mainly from coniferyl alcohol (over 95% guaiacyl structural element), while hardwood ones are called GS lignins and are essentially made up of coniferyl and synaphyl alcohol. Lignins from non-wood materials have varying amounts of the three units (HGS) (24).

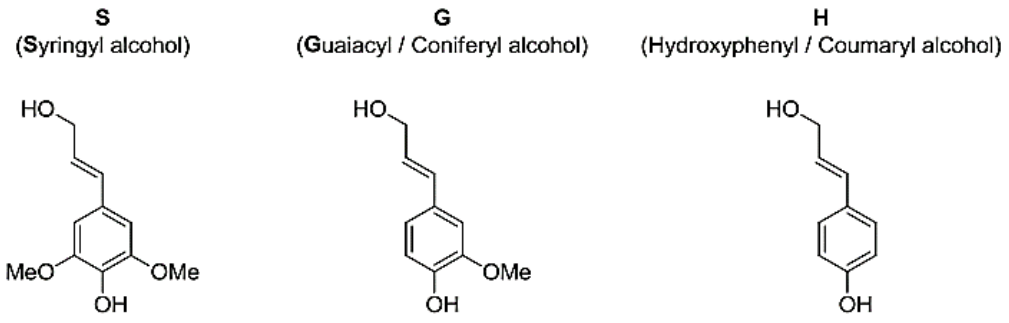

Figure 1-3-Three important structures of lignin **S**: sinapyl alcohol, **G**: coniferyl alcohol, and **H**: *p*-coumaryl alcohol. (24)

The molecular mass of isolated lignins diverges, making it difficult to quantify the degree of polymerization as it exists in nature, due to the inevitable fragmentation that occurs during its extraction. Although the existence of physical and chemical interactions between lignin and polysaccharides (hydrogen bonds, van der Waals forces, and covalent bond) is evident, the nature and number of these bonds are not exactly known. Lignin is linked to a part of the hemicelluloses, with covalent ether or glucoside bonds being the most frequently suggested (25, 26).

In the degradation processes of lignocellulosic, the recalcitrance of the lignin polymeric structure is well known. Acid and alkaline depolymerization of lignin will result in breaking of the ester bonds and some of the ether bonds, but the reactivity of the liberated fragments may result in a rearranged and even more condensed polymeric structure (Figure 1-4). Therefore, the extraction conditions that are applied to lignocellulosic biomass substantially affect the structure and properties of the resulting (technical) lignin (24).

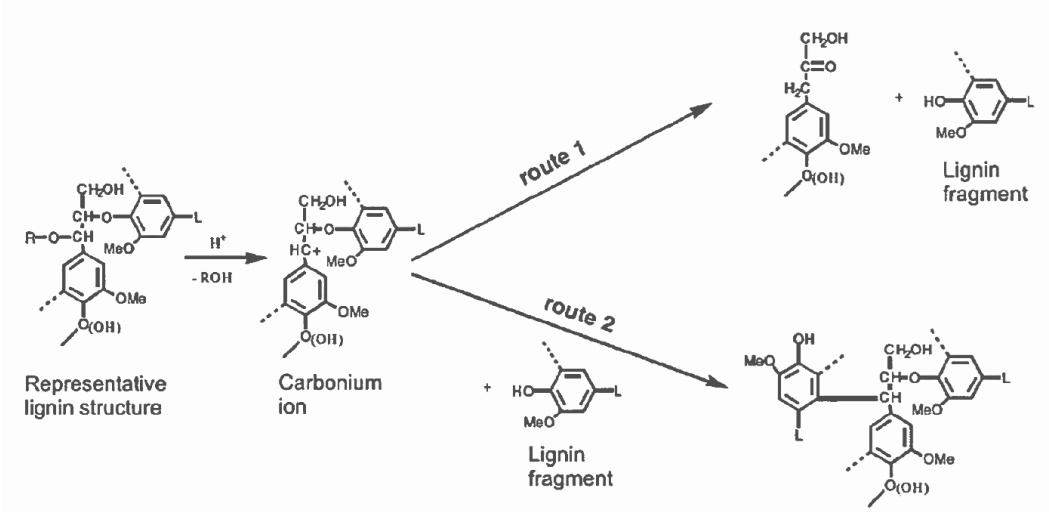

Figure 1-4- Competition between depolymerization of a β-O-4 structure (Route 1) and repolymerization involving a lignin structure (Route 2) (24)

The structure of lignin is quite complex, and its composition depends on the origin of the raw material and varies according to the extraction technique used (Figure 1-5).

Due to its chemical nature, lignin is one of the natural polymers most resistant to alkaline, acidic and enzymatic hydrolysis reactions, making it more susceptible than polysaccharides to oxidation reactions or the action of organic solvents (27).

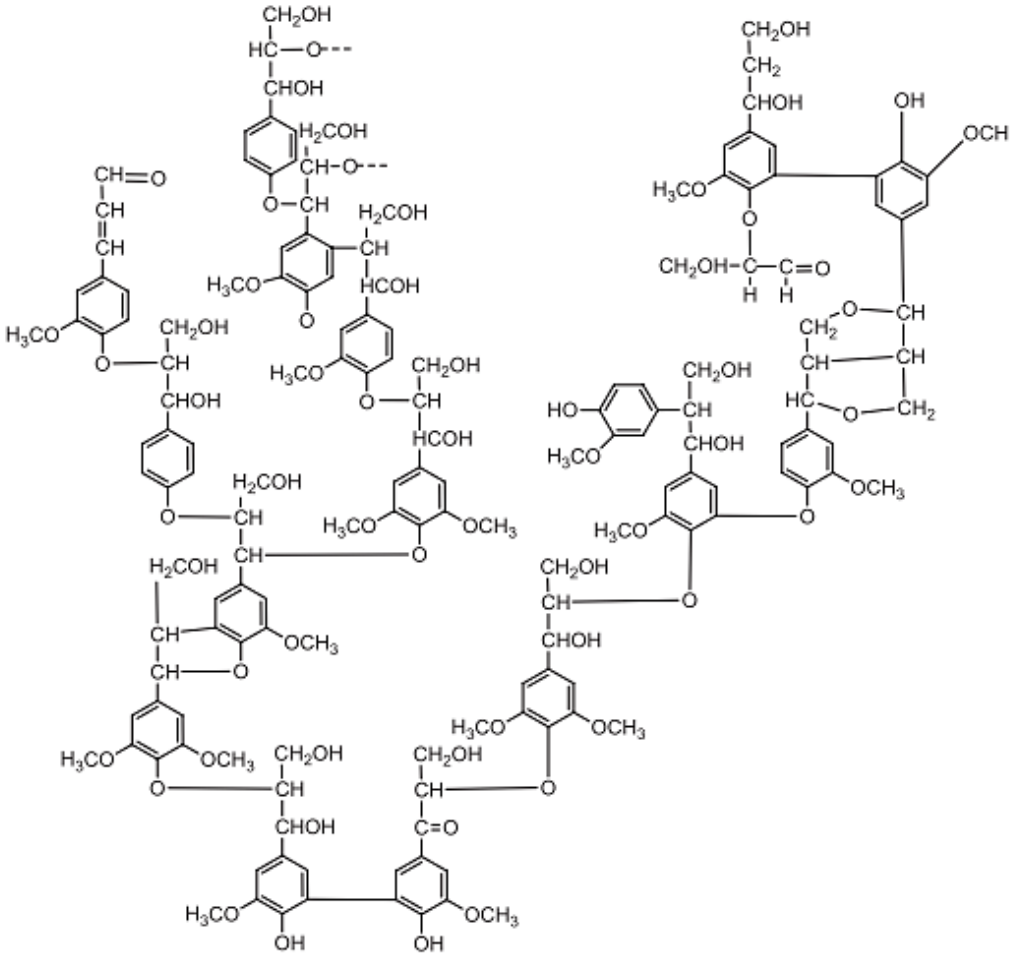

Figure 1-5- Structural model of spruce lignin purposed by (28)

1.2.3 Pre-treatment/fractionation review

The biomass pre-treatment phase has evolved during the last years. The main interest to use lignocellulosic materials shifted from an exclusive bioethanol production to a better awareness of the other main compounds, lignin, and hemicelluloses. This new vision finds ways to maximize the overall yield of the compounds that make up lignocellulosic materials. Pre-treatment methods that allow efficient recovery of carbohydrates, as well as lignin, are desired depending, always, on the final product (Figure 1-6).

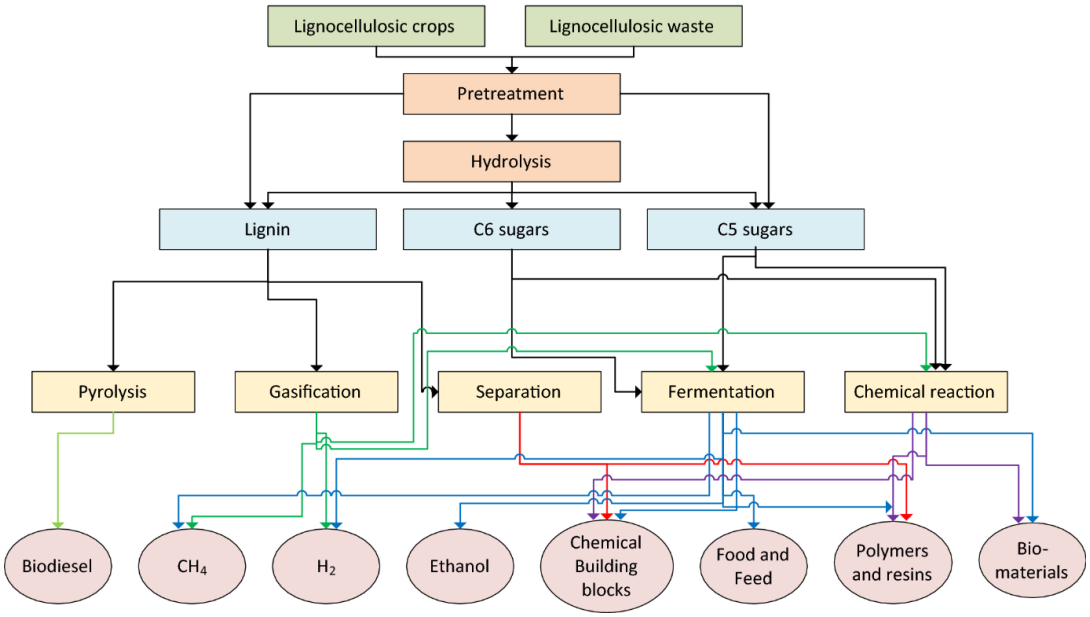

Figure 1-6-Products from lignocellulosic biomass (29)

Pre-treatment is one of the first steps in the process, to ease access to the raw material, being vital that some important features of the pre-treatment method are fulfilled, such as a high recovery of the individual polymers and other compounds in the lignocellulosic material. Moreover, the growth of toxic or inhibiting compounds must be the minimum possible to decrease the risk for negative effects during the next steps, as the enzymatic hydrolysis and fermentation steps, if they are needed.

An effective pre-treatment should disrupt the hydrogen bonds of crystalline cellulose, breakdown the matrix of lignin and hemicelluloses, and increase the surface area and porosity of cellulose for enzymatic hydrolysis (1). Delignification increases enzymatic digestibility of substrate since it eliminates a physical barrier to cellulolytic enzymes and because lignin/phenolic compounds can non-productively adsorb those enzymes, acting as inhibitors to enzymatic hydrolysis (30). The application of too severe conditions during pre-treatment will cause superior degradation of hemicellulosic sugars, which can origin the formation of toxic compounds, such as furfural, HMF, and organic acids. Additionally, other compounds may be generated; however, furfural and HMF are often used as representations for the general content of inhibitory compounds (31).

Pre-treatment represents a considerable portion of biomass processing costs (20-40%) (32) and has a great influence on economic and environmental performance. The developments in pre-treatment approaches are widely recognized as a crucial way to improve the economic competitiveness of biomass conversion (33).

Fractionation methods can be divided into physical, chemical, physicochemical, and biological processes (Table 1.1). Even if some of these methods are already well established, they still have limitations that need to be studied. Some important progress has been made, not only in the optimization of these processes but also in terms of the development of other alternative and innovative processes that explore different properties of lignocellulosic materials (34).

Table 1.1-Comparison of some lignocellulosic biomass fractionation processes (adapted from (1, 26, 34))

	Physical	Chemical						Physicochemical				Biological
		Acid			Alkaline	Organosolv	Ionic liquids	AFEX	LHW	Steam explosion	CO_2 Explosion	
		Dilute	Concentrated	Solid acids								
Temperature	↓	↑	↓/0	0	↓/0	0/↑	↓/0	0	↑	↑	0	↓
Hemicellulose removal	↓	↑	↑	US	0	↓/0	↑	↓	↑	↑	↑	↓
Hemicellulose recovery	↓	↑	Moderate	US	0	↓	↑	↓	↑	0	↑	↓
Cellulose Hydrolysis	↓	↓	↑	US	↓	↓	↓/0/↑	↓	↓	↓	↓	↓
Enzymatic digestibility	0	↑	N.A.	US	↑	0/↑	↑	↑↑	↑	↑↑	↑	0
Lignin removal	↓	↓	↓	↓↓	↑	↑↑	↑	↓	↓	↓	↓	↑
Inhibitors formation	↓	↓/0	↓/↑	↓	↓	↓	↓	N.A.l	↓	↓/0	↓	↓
Equipment corrosion	↓	0	↑	↓	↑	0	↓	↑	↓	↓	↓	↓
Energy required	↑	↑	↓	↓/0	↓	0/↑	0	↑	↑	↑	↑	↓
Catalyst recovery	N.A.	Difficult	Required	Easy	Easy	Required	N.A.	Required	N.A.	N.A.	Required	N.A.
Residues formation	↓	↑	↓	N.A.	↓	↓	N.A.	N.A.	↓	↓	↓	0
Implementation at pilot scale	Yes	Yes	Yes	No	Yes/No	Yes	No	No	Yes	Yes	Yes	No

↑ - high; ↓ low; 0 – moderated; US – under study; N.A. – not applicable

1.2.3.1 Physical/Mechanical pre-treatments

Mechanical pre-treatments have the purpose of reducing particle size, offering many advantages. These include: i) increasing the the surface area of biomass, ii) simplification of densification processes, iii) simplification of the supply chain of raw materials and their storage conditions, iv) increasing of the total accessible surface area, v) improving the bio-accessibility of constituents, vi) improving conversion of saccharides during hydrolysis, vii) reducing the mass and heat transfer limitations during the hydrolysis reactions, and finally, viii) reducing the energy inputs.

Different types of size reduction are usually distinguished, like cutting or crushing, coarse milling, intermediate micronization, fine grinding, ultra-fine grinding, and nano-grinding (Figure 1-7). These can be achieved through a combination of diverse mechanical stresses such as, impact, compression, friction, and shear. For instance, in a jet mill, impact and friction between particles are created when the particles are projected against each other in an air stream (1, 35).

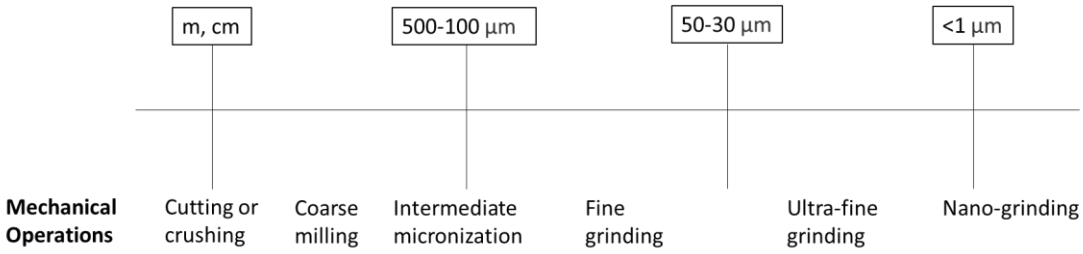

Figure 1-7- Mechanical operations for size reduction, adapted from (35)

The fragmentation and dissociation of lignocellulosic biomass can be achieved using different kinds of mill tools like a knife mill, hammer mill, pin mill, and centrifugal mill. The working principle of these tools of a rotor driving different tools, where the rotor speed is usually adjustable, and resorting to a sieve or a screen the final size of the particle can be controlled. These mills generate more impact and shear, however in ball mills including a vibratory ball mill and tumbling ball mills, the raw materials suffer impact and compression stresses when collisions between balls and walls occur. Lastly, in an extruder, the dynamic between the screw and the walls provokes shear, being this the main mechanical stress in this kind of operation. The choice of equipment depends on many parameters like physical and chemical properties of the biomass, the moisture content, final targeted particle size, particle size distributions, and application.

The energy requirement depends on machine specifications such as motor speed, the storage capacity of the milling chamber, material throughput characteristics, initial biomass structure, and physical-chemical proprieties (moisture content, chemical composition, tissue composition, pos-treatment) and initial and final particle sizes. Typically, these methods are applied previously or simultaneously to other pre-treatments, as their intensive energy requirements make them economically unsuitable to be used on their own (1, 35).

1.2.3.2 Physicochemical/Hydrothermal treatments

Hydrothermal treatments of biomass are based on the use of water, steam, or both and heat. In these conditions, hydrolysis of the acetyl groups of the hemicelluloses occurs, with the solubilization of the hemicelluloses, leaving cellulose more accessible, for example, for later enzymatic hydrolysis (15, 36). The main difference of these processes concerning the hydrolysis with dilute acid is that the hemicelluloses are mostly recovered in the oligomeric form, whereas in the processes that use acids, essentially, monosaccharides are obtained. Among the hydrothermal methods, two main ones are distinguished: liquid hot water (LHW) and steam explosion. Within the group of physicochemical treatments, there are still AFEX and CO_2 explosion that applies the principles of steam explosion, but the vaporized substances are not water.

1.2.3.2.1 Liquid hot water/ Autohydrolysis

The liquid hot water method is based on the use of compressed water at high temperatures, between 130 and 230ºC, with a variable reaction time from seconds up to hours, depending on the temperature. The hydrolysates obtained are essentially composed of hemicellulose derivatives and a solid residue composed mainly of cellulose and lignin (37). This method can solubilize until more than 80% of total hemicellulose, without affecting the more resistant crystalline cellulose structure.

The underlying mechanism of LHW is auto-hydrolysis since the catalysts are primarily H_3O^+ ions from the auto-ionization of water and in a later stage H_3O^+ ions from the acetyl groups of the hemicelluloses (38). Uronic acids can also contribute to the release of hydronium ions but their effects are not well established (39). The heterocyclic ether unions of the hemicelluloses are the most susceptible to this type of reaction and their rupture leads to the formation of oligosaccharides with different degrees of polymerization.

Autohydrolysis only uses water, which brings many advantages such as reduced corrosion problems (7, 40), and the lack of the necessity to recycle acids as well as the removal of precipitates. This simplified process leads to a reduction of operating capital costs, which provides economic benefits, and reduced the environmental impact of aqueous treatments comparing to other technologies. (7, 40, 41). However, this process is yet not developed at a commercial scale because of higher water demand and high energy requirements. Additionally, LHW has a limited effect in biomass deconstruction originating, typically, low enzymatic hydrolysis yields (42).

1.2.3.2.2 Steam explosion

Steam explosion is a process in which biomass is treated with high pressure saturated steam at temperatures approximately 160–260 ºC and pressure in the range of 0.7 and 4.8 MPa. The pressure is held for several seconds to a few minutes to promote hemicellulose hydrolysis, followed by mechanical disruption of the pretreated material by violent liberation into a receiving reservoir (43). The explosion quickly disrupts the linkages between cellulose, lignin, and hemicellulose and some of the hexoses and pentoses from hemicellulosic fraction are degraded into aldehydes (HMF and furfural) and

organic acids, which are potential inhibitors during fermentation of the sugar fraction. This leads to the necessity to wash the pretreated biomass with water to remove the toxic materials along with water-soluble hemicellulose (44). The high-pressure steam drastically modifies the plant cell wall structure, yielding a water-insoluble fraction composed of cellulose, residual hemicelluloses, and a (chemically modified) lignin that can be further extracted by a subsequent alkali extraction, or may be recovered and used for the production of various chemicals (43, 45). The liquid fraction is rich in monomeric and oligomeric sugars. Highly digestible cellulose or high yields of solubilized hemicellulosic sugars can be achieved under optimized conditions. Processing the solid fraction with enzymatic hydrolysis, most of the cellulose is commonly converted to glucose, leaving behind lignin that has high heating value and can be burned for process energy or converted to pellets, which can be sold to improve process economics (46).

Steam explosion is considered as the most effective pre-treatment for hardwood and agricultural residues, however not so good for softwoods (47). This pre-treatment has several potential advantages, like lower capital investment, lower environmental impact, and less hazardous process chemicals and conditions compared with other pre-treatments (46, 48, 49). Moreover, it offers a reduction of energy consumption by more than 40% when compared with conventional mechanical methods used to attain the same size reduction (47). These advantages are proof of the potential applications of this method at the industrial scale. However, there are several disadvantages as well, like the incomplete deconstruction of the lignin carbohydrate-complex and the condensation and precipitation of soluble lignin components on pretreated solid surfaces, which results, sometimes, in the low efficiency of enzymatic hydrolysis. Additionally, a portion of the hemicellulose constituent of the biomass may degrade, leading to the generation of inhibitors, as mentioned before.

1.2.3.2.3 Ammonia fiber explosion (AFEX)

AFEX follows the same process concept as the steam explosion, but instead of water, it uses ammonia as the vaporized substance. The optimization of this pre-treatment is done controlling four parameters including ammonia loading, water loading, reaction temperature, and residence time. Typical operation conditions are 60-120ºC and 17-21 bar for less than 30 min. AFEX produces only solid material due to the ammonia's low boiling point (ammonia vaporizes entirely) and does not liberate any sugars directly because of low hemicelluloses solubilization, in contrast with the steam explosion. It opens the structure of lignocellulosic biomass and increases the polymer's surface area favoring the enzymatic digestibility. AFEX pre-treatment has been demonstrated to result in higher conversion rates of different kinds of cellulosic biomass, such as aspen wood, WS, alfalfa stems, switchgrass, rice straw, corn stover, and is less effective on high-lignin content biomass such as hardwood and softwood feedstocks, which present higher lignin contents, that are more recalcitrant to AFEX pre-treatment. To reduce the high operation cost basically due to the high cost of ammonia as well as environmental issues, and efficient ammonia recovery and recycling is mandatory (1).

1.2.3.2.4 Carbon dioxide explosion

Carbon dioxide explosion follows the same principles as steam explosion where super-critical CO_2 (31ºC, 74bar), a substance with a liquid-like density while exhibits gas-like transport properties of diffusivity and viscosity, is used instead of water or ammonia. CO_2 molecules have a similar size property to those of water and ammonia making them capable of penetrating small pores of lignocellulosic material. Supercritical CO_2 explosion has the advantages of needing lower temperature and having lower toxicity and flammability in comparison with the steam explosion and AFEX (50). CO_2 is a cheap gas, representing fewer costs than ammonia for AFEX (42) and has the advantages of an acid-catalyzed process by forming carbonic acid when CO_2 is dissolved in water, but with significantly less corrosiveness due to the specific features of carbonic acid. Also, due to the easy recovery of CO_2 by depressurization, it generates no waste products (1).

1.2.3.3 Biological pre-treatment

Biological pre-treatment work principle consists in the conversion of lignocellulosic biomass by microorganisms, especially fungi, into more accessible compounds, with no chemical requirements (51). This method has some advantages comparing with chemical and physicochemical pre-treatments like requiring low capital and operational cost since it takes advantage of white-, brown-, soft-rot fungi to delignify and enhance enzymatic hydrolysis of lignocellulosic biomasses. Despite the low energy consumption, environmentally friendly conditions, and no chemical requirement, biological pre-treatment still faces some weaknesses affecting its widespread application as a commercial pre-treatment method, such as long process times, large space requirements, and the need for continuous monitoring of microorganism growth (1).

1.2.3.4 Chemical pre-treatment

1.2.3.4.1 Alkaline pre-treatment

The alkaline treatment, where the biomass is processed with alkali, is explored based on its main advantage that is the efficient removal of lignin from the biomass as it is very soluble in alkaline solutions due to its high hydroxyl content. This process can largely improve the cellulose digestibility, exhibiting minor cellulose, and hemicellulose solubilization compared to acid pre-treatment (7). This process also removes acetyl and uronic acid groups present on hemicelluloses, which consequently enhances the accessibility of the enzyme that degrades hemicellulose. Also occurs the saponification of intermolecular ester bonds cross-linking xylan hemicelluloses and other components, thus the porosity of the lignocellulosic biomass increases with the removal of the cross-links. This pre-treatment has the advantage of using lower temperatures (30-130ºC) and producing fewer degradation products than acid hydrolysis. Although to achieve the necessary yields, long residence times (hours to days) and slurry neutralization are required. Alkaline pre-treatments are very costly due to the necessary downstream processes for the recovery/neutralization of alkalis and the large amounts of washing water used (42).

The most used alkalis are sodium (soda) or potassium hydroxide, calcium hydroxide (lime), and ammonia (1), with soda being the most effective. Lime, despite being less effective, is a cheaper option, poses fewer safety hazards compared to soda and potassium hydroxide, and is easier to recover via reaction with carbon dioxide. Alkaline pre-treatment is reported to be more effective for non-woody feedstocks, such as agricultural residues The addition of air/oxygen to alkaline pre-treatment using NaOH or $Ca(OH)_2$ can improve the treatment efficiency by increasing lignin removal (7).

1.2.3.4.1.1 Kraft Process

The two main alkaline delignification processes are the soda process and the Kraft process. The two processes are similar since both use sodium hydroxide as the main delignification reagent, though the kraft process applies an additional component, sodium sulfide. The Kraft process is a traditional method in the pulp production process due to its efficiency and easily adaptability to several types of wood, allowing the production of good quality pulp. Using sodium hydroxide and sodium sulfide under strong alkaline conditions the lignin's C-O-C bonds are cleaved resulting in a liquor rich in phenolic compounds called black liquor that is the process effluent. Lignin is usually recovered from black liquor by acid precipitation (52). This delignification process is usually carried out at temperatures between 150ºC and 170°C, although higher cooking temperatures can be used. During this treatment, several reactions occur and, in addition to lignin, part of the hemicellulose dissolves. Lignin is fragmented essentially by the action of hydroxyl ion (OH^-) and hydrosulfide (SH^-), producing lignin with aliphatic thiol groups called Kraft lignin.

The main advantages of this process are low demands on wood species and quality, short cooking times (4h-6h), well established processing of the spent liquor (recover of the pulping chemicals, generation of process heat, production of valuable by-products) and exceptional pulp properties (21). However, this process discharges huge amounts of liquid and gaseous wastes such as sulfur-containing gas emissions, making it a non-environmentally friendly process (7).

1.2.3.4.2 Ionic Liquids pre-treatment

Ionic Liquids are organic salts composed of cations and anions, typically, large organic cations and small inorganic anions, with a melting point lower than 100ºC. The main factors responsible for the interaction between the ILs used and the lignocellulosic materials are cations, anions, temperature, and time used during the pre-treatment. The combination of different cation and anion consent great versatility to ILs, giving them different properties and behaviors when effecting the treated biomass. Recently, imidazole-based IL has received much attention due to their remarkable cellulose dissolution capability (53).

ILs have the advantage of the rapid dissolution of lignocellulosic materials at mild temperatures, but not at a complete level (41), less dangerous process conditions and chemicals, green solvents due to low vapor pressure, high thermal and chemical stability and ionic liquids are easily recycled. However, this pre-treatment has some drawbacks in the application at industrial scale like incompatibility of IL with cellulase leading to the inactivation and unfolding of the enzyme, huge amounts of currently

expensive ILs is needed, the high energy requirements associated with their recycling and the increased viscosity of the reactional mixture during pre-treatment, that makes it difficult to handle (1).

1.2.3.4.3 Organosolv pre-treatment

Organosolv is a process that uses a mixture of organic solvent and water, at high temperatures, to remove hemicellulose and lignin from biomass. From this pre-treatment two fractions are obtained: a solid fraction enriched in cellulose and containing some non-solubilized hemicelluloses and lignin, and a liquid fraction with the solubilized sugars (hemicelluloses in the oligomeric and monomeric form) and lignin (acid-soluble lignin), as well as some degradation products, organic acids, extractives, and soluble ash. The main advantages of using the organosolv process are the deconstruction of lignocellulose increasing solvent penetration and biomass dissolution, enhancing hydrogen transfer, reaction kinetics (by decreasing its activation energy) and the possibility of isolating the components (cellulose, hemicellulose, and lignin) with a relatively high degree of purity and integrity, and the possibility of solvent recovery and reuse. Some solvents that have already been evaluated for the organosolv process, for example, ethanol, acetic acid, and glycerol are already being produced from renewable sources, favoring the sustainability of the processes in which they are used. Ethanol is the most used and studied solvent, due to its low cost, low toxicity, miscibility in water, and ease of recovery. However, on a pilot and commercial scale, other solvents, such as methanol, acetic acid, and formic acid have also stood out. In addition to the characteristics described above for ethanol, it is expected that the solvent chosen for the biomass fractionation process does not cause mass transport limitations, does not promote undesired reactions with biomass components and equipment, and favors the reactions of interest for reducing the activation energy. Yet, the volatility and flammability of organic solvents constitute safety and environmental hazard and increase the operation and capital costs due to the high pressures applied. Moreover, another disadvantage is the need for recycling the solvent, necessary to lower operation costs and prevent the inhibitory effect on enzymatic hydrolysis and fermentation processes (54, 55).

The number of steps in the organosolv process for obtaining isolated biomass components can vary significantly depending on the type of biomass, the solvent used, and whether the process is continuous or batch. The reduction in the number of steps, without losing the quality of the products in this process, is the biggest challenge to achieve economic viability (56).

1.2.3.5 Acid Hydrolysis

1.2.3.5.1 Homogenous Acids

In homogenous acid hydrolysis, the most used catalyst is sulfuric acid (H_2SO_4), although other inorganic acids, such as hydrochloric acid (HCl), nitric (HNO_3) and trifluoroacetic (TFA), can also be used (7, 41). This pre-treatment can be separated in two kinds since lignocellulosic materials can be hydrolyzed with dilute acid at elevated temperatures, which solubilize almost all hemicelluloses, or using concentrated acid at moderate temperatures that cause the total hydrolysis of cellulose and hemicelluloses, with lignin remaining as an insoluble residue (57).

The hydrolysis mechanism of the hemicelluloses in an acidic medium can be represented by three main steps (Figure 1-8). The oxygen protonation of the glycosidic bond occurs, followed by the rupture of the glycosidic bond with the formation of a carbocation and eventually, regeneration of the H_3O^+ ion occurs, establishing a final stable molecule, by the reaction of the carbocation with the water molecule.

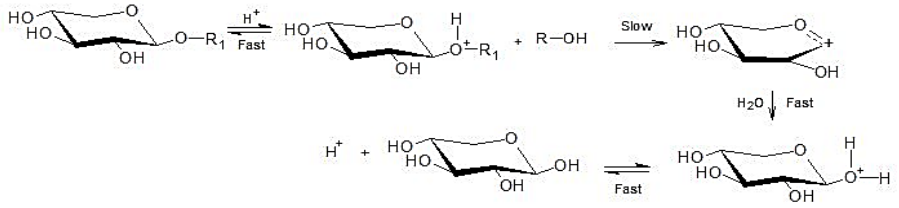

Figure 1-8- Mechanism of acid hydrolysis of hemicellulose (58)

In the case of cellulose, the formation of the carbocation is hampered by the intramolecular hydrogen bonds of the cellulose chains. This leads to the hydrolysis of the amorphous areas of cellulose occurring much faster than in the crystalline areas (36). Since hemicelluloses do not have a crystalline structure, they are expected to be solubilized much more easily than cellulose. Thus, due to these differences between cellulose, hemicelluloses, and lignin, it is possible to select the operating conditions so that hydrolysis is more selective, depending on the objectives that are intended.

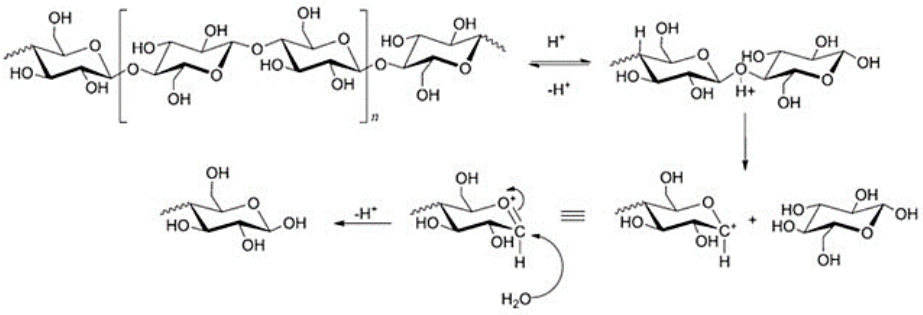

Figure 1-9- Mechanism of acid hydrolysis of cellulose (59)

1.2.3.5.1.1 Concentrated acid

This type of process generally occurs at moderate temperatures, 20-50ºC, and in relatively short times, 20-60 minutes, and it allows the solubilization of polysaccharides using different acid concentrations, for example, 72% H_2SO_4, 41% HCl or 100% TFA. The last two acids have the advantage of being easily recoverable. Concentrated acids allow cellulose and hemicelluloses to be solubilized, obtaining a solid residue consisting essentially of lignin. Concentrated acids can attack hydrogen bonds between cellulose chains, destroying their crystallinity. Despite the formation of reduced quantities of degradation products and the possibility of operating at low temperatures and pressures, the costs involved in neutralizing the hydrolysates, acids recovery (mandatory for economic

viability) and the problems associated with equipment corrosion, make this process disadvantageous to hydrolysis with dilute acids and hydrothermal methods (41, 60).

1.2.3.5.1.2 Diluted acid

Diluted acid hydrolysis is probably the most widely used chemical pre-treatment method in the scientific literature. This can be used, for example, as a pre-treatment method for lignocellulosic biomass before enzymatic hydrolysis of cellulose. However, it is not efficient in dissolving lignin, but can affect its structure and increase the susceptibility of cellulose to enzymatic hydrolysis. This method is also effective in the selective hydrolysis of the hemicellulosic fraction to obtain monosaccharides, presenting as advantages the possibility of, in controlled conditions, avoid the formation of undesirable products and equipment corrosion. To the fermentation of the hydrolysates be possible, it is necessary to proceed to the neutralization and/or detoxification of the hydrolysates. In the literature, it is possible to observe a great variety in the hydrolysis conditions with diluted acid for a great diversity of lignocellulosic biomass. The differences are fundamentally found in the type of acid used, as well as in its concentration, temperature, and duration of the hydrolysis reaction. (7, 61)

1.2.3.5.1.3 Solid acids

Examples of solid acids studied for lignocellulosic biomass pre-treatment include niobic acid (Nb_2O_5 nH_2O), H-mordenite (zeolite), Nafion NR50 (perfluorosulfonated ionomer), Amberlyst-15 (polystyrene-based cation-exchangeable resin with SO_3H groups), sulfonated activated-carbon, and amorphous carbon bearing SO_3H, COOH and OH, as well as other materials such as bentonite, kaolin and acid-treated alumina. The later materials and amorphous carbon bearing SO_3H, COOH and OH have been reported to effectively treat the solid biomass at relatively mild temperatures (100 ^{O}C) (41).

1.2.4 Hydrolysis of hemicellulose-derived oligosaccharides

Renewable bioresources like lignocellulosic biomass are converted by chemical companies into biofuels, fine chemicals, agro-chemicals, and specialty chemicals such as bio-lubricants, natural fibers and bio-based solvents (62). Several products derived from renewable resources such as ethanol, glycerol, lactic acid, succinic acid, levulinic acid, are already in use or considered with potential importance in the near future.

The hemicellulosic fractions can be the source of some of these products (Figure 10), i.e. furanic compounds, such as 2-furaldehyde (furfural), obtained from the degradation of hemicellulosic pentoses, has various industrial applications (63), and xylitol and butanol, both produced by fermentation of hemicellulosic hydrolysates, are also marketable products. Ethanol can also be produced from hemicellulosic hydrolysates although, few microorganisms can produce it efficiently (41). Figure 1-10 shows some hemicellulose derived products.

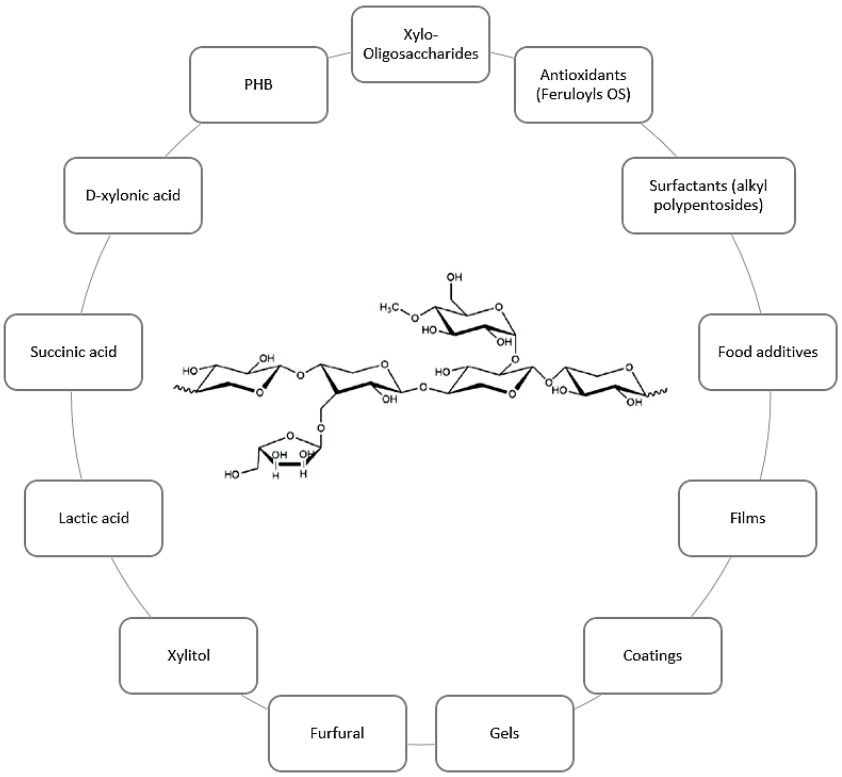

Figure 1-10-Hemicellulose derived products

Of the promising pretreatment options, dilute acid is yet the most developed. Hemicellulose recoveries are much higher than other pre-treatments. Dilute acid pretreatment also produces less fermentation inhibitors, and significantly increases the later cellulose hydrolysis. However, the acid consumption is an expensive part of the method's costs, gives a waste disposal problem and requires the use of expensive corrosion resistant materials. Furthermore, lignin valorization potential is degraded.

This may eventually tip the balance in favour of the less effective, but also less problematic and environmentally friendly autohydrolysis or organosolv. However, autohydrolysis and organosolv lead to oligomeric saccharides (34), which have to be converted into monosaccharides before fermentation. The post-hydrolysis options are reduced to acid or enzymatic hydrolysis.

The main advantage of enzymatic post-hydrolysis over the acidic process is the milder operation conditions, which leads to reaction media free of sugar- or lignin- degradation compounds that can limit microbial performance. Conversely, the acid process is faster; the catalyst is cheap and allows high monosaccharide recovery.

The most important enzyme activities for xylo-oligosaccharides hydrolysis (7) are summarized in Table 1.2.

Table 1.2-Relevant enzymatic activities for the enzymatic hydrolysis of xylooligosaccharides.

Enzyme	EC	Hydrolysed Linkage	Substrate	Main product
Endoxylanase	3.2.1.8	Internal β-1,4	Main chain	Oligomers
Exoxylanase	n.c.	Terminal β-1,4 (reducing end)	Main chain	Xylose, xylobiose
β-Xylosidase	3.2.1.37	Terminal β-1,4 (non-reducing end)	Oligomers	Xylose
α-Arabinosidase	3.2.1.55	Terminal α-1,2; α-1,3	Side groups	Arabinose
α-Glucuronidase	3.2.1.139	Terminal α-1,2	Side groups	Methylglucuronic acids
Acetyl xylan esterase	3.1.1.72	Ester bond	Side groups	Acetic acid
Feruloyl esterase	3.1.1.73	Ester bond	Side groups	Ferulic acid

1.2.4.1 Heterogenous Acids

The definition of a solid acid catalyst is a solid that can donate protons or accept electrons during reactions. The catalytic function for a solid acid catalyst results from its acidic centers, existing mainly on its surface. Consequently, solid acids with Brønsted acid sites can catalyze biomass hydrolysis (64). These solid acids are even considered superacids, a name given to a system that is stronger than 100% sulfuric acid (65), which is already a strong acid due to its ability to be completely dissociated or ionized in an aqueous solution (High capacity to lose a proton, H^+) (66). Strong acids have a small logarithmic constant (pKa) due to a large acid dissociation constant (Ka).

In an integrated biorefinery framework, the hydrolysis of hemicellulose-derived poly/oligosaccharides into monomeric sugars is vital, but process options are restricted to the use of homogenous acid (H_2SO_4) as enzymatic processes are still underperforming (41). However, solid acid catalysts can be a potential replacement for mineral acids since they have some inherent advantages. Heterogeneous catalysts can easily be separated, recovered, reused and they are safe and non-corrosive (64). The use of solid acids keeps the advantages of using acid hydrolysis but improves selectivity towards the glycosidic bonds at the expense of the dehydration of monosaccharides allowing a higher sugar yield. Moreover, the hydrolysis of soluble oligosaccharides over a solid acid catalyst can be performed in a dynamic flow reactor, being possible the implementation of a continuous process and thus decreasing production cost (72).

Cellulose is a solid polysaccharide with a crystalline structure making it very difficult to solubilize and hydrolyze. On the other hand, hemicellulose's oligosaccharides are soluble compounds and are relatively easy to hydrolyze with solid catalysts as there is no solid-solid transfer involved. Therefore, the catalysts and the reaction conditions used in both cases are different and the results obtained on cellulose hydrolysis cannot be transposed to hemicellulose's oligosaccharides hydrolysis. The most often proposed pathway for the valorization of pentoses is dehydration into furfural, which is a building block for the synthesis of fuels and chemicals (67). However, further interesting and valuable products such as xylitol can also be produced from pentoses. That is why hydrolysis of oligosaccharides into

pentoses and hexoses without further dehydration into furfural and HMF is a great advantage for numerous processes (68).

Despite the advantages mentioned before, it is a challenge to develop hydrothermal catalytic hydrolysis processes with solid acid catalysts. First, their acid strengths and catalytic activities decrease when water is present. Second, most solid acids do not function effectively for biomass hydrolysis because the surfaces of these solids do not have strong acid sites. As a result, a good solid acid catalyst needs to be water-tolerant, have strong acidity, and have many acid sites available (69).

There are several different groups of potential solid acid catalysts.

1.2.4.1.1.1 Zeolites

Zeolites are crystalline aluminosilicates with complex tridimensional structures creating different levels of porosity: mesopores (5–50 nm) and micropores (<5 nm). Their surfaces, acid strength, and acid site concentrations can be finely tuned by several preparation methods. However, this kind of solid acid catalyst presents stability problems in the presence of water. Reports are claiming that faujasite H-Y zeolites deactivate in a range between 160ºC and 200ºC, in liquid water for a few hours (70, 71). The H-USY is a type of zeolite treated by steaming to improve the stability that could overcome this issue. These materials can hydrolyze xylan in the water at 140ºC. Zeolites can convert disaccharides into monomer sugars at temperatures between 80 and 140ºC. Theoretically, they can catalyze dehydration reactions such as fructose dehydration into HMF, however, in most cases the yield in dehydration products is low. The Si/Al ratio is an essential property of zeolites. When the Si/Al ratio increases, the number of Brønsted acid sites decreases, and the average acid strength increases. The adsorption of the reactant seems to be favored by decreasing aluminum content (68).

1.2.4.1.1.2 Ion Exchange Resins

Ion Exchange Resins (IER) are organic polymers synthesized as beads that can contain a macroreticular structure with an important specific surface area and macroporosity. Their high ion-exchange capacity is used for the adsorption of cations. Due to the presence of sulfonic groups (SO_3H) resins are also used in organic synthesis as acid catalysts. One of the most common resins is Amberlyst-15. They have the advantage of converting disaccharides into monomeric sugars at low temperatures (40–130ºC). At 80–90ºC, for example, they are more active than zeolites (68). Their adsorption and ion-exchange properties can also be used to remove impurities before the fermentation process, such as cations, proteins, furfural, HMF, aliphatic acids, or lignin derivatives (68).

The solid nature of the IER allows mechanical separation of the catalyst and the reactant-product mixture by filtration or decantation, eliminating distillation or extraction procedures for product isolation. Resins can be used as catalysts in continuous processes in fixed bed reactors, yet the particles should preferably be of uniform size because smaller particles may fill up space between larger particles causing the improper distribution of the reactants in the resin bed.

In terms of economic viability, IER presents some potential advantages. Catalysis by IERs lowers capital and processing costs by eliminating the steps and equipment required for catalyst removal in analogous homogeneous processes, and the waste disposal problem is avoided. Resins

can often be used, in hundreds of catalytic cycles without regeneration in well-designed processes. The cost of the resin is spread over the life of the resin, making the catalyst cost per unit production lower than their homogeneous competition.

IER even having the equivalent strength of strong mineral acid, are safe to handle. Due to its heterogeneous nature the resin, though it contains acid at high concentration, can be used conveniently in mild steel equipment. The number of acid groups at the surface of the bead in contact with equipment is a very small percentage of the total number of acid groups present, lessening the problem of corrosion. The major disadvantage of IERs in catalysis is their low thermal stability. Typically, IERs cannot withstand temperatures higher than 125°C for long periods (72). This can be a major issue for sugars hydrolysis in which the temperature can be close to 130ºC or above. However, there are reports that Amberlyst-35W could be stable until 160ºC despite the commercial recommendation (72). Nafion can withstand temperature up to 200°C, but it is a very expensive material. Another disadvantage is, for macroreticular resins, operation at 150°C for a long time may cause desulfonation leading to a release of sulfonic acid as well as a drop in the activity. (73)

This thesis focused their experiments on this kind of solid acids, so deeper research was performed in the ion exchange process and resins. More details are given in Annex 3: Ion Exchange.

1.2.4.1.1.3 Carbon materials

Activated carbon, char, or carbon nanotubes are carbon materials with very high specific surface areas (> 500 m^2 g^{-1}), with an important microporous volume, used as catalysts or sorbents.

As heterogeneous catalysts, these materials are used as metal supports or as acid catalysts. In the latter case, they can be acid-treated to form sulfonic (SO_3H) groups at the carbon surface. The carbon materials with SO_3H groups can be prepared by incomplete carbonization of sulfoaromatic compounds or sulfonation of incompletely carbonized organic matter. While the former can be used to prepare carbon material with a high density of SO_3H groups (>4 mmol g^{-1}) (74), there are several problems associated with large scale production and safety, because sulfoaromatic compounds are carbonized in concentrated H_2SO_4 above 200ºC. The second route is safer and easier to prepare, although the amounts of acid are smaller than the first route (<2.5 mmol g^{-1}). In this preparation, sugars, starch, cellulosic materials, and polymers, anything that can be carbonized, can be used as starting materials. The carbonization temperature controls the catalytic performance of the material prepared by this method. Low carbonization temperatures (<250ºC) only provide complex polymers containing aromatic compounds. Sulfonation of such pyrolysates results in soluble sulfonated species. In contrast, high carbonization temperatures (>450ºC) greatly decrease catalytic performance due to the growth of the carbon network (74).

Sulfonated carbons and chars were reported to have good performances as catalysts in the hydrolysis of various disaccharides. Their hydrophilic behavior, due to hydroxyl surface groups, could explain their high activity since the weak acid sites present on the carbon surface (hydroxyl and carboxyl groups) easily adsorb the carbohydrates. Reports are claiming that chars prepared from biomass showed a higher activity for cellobiose hydrolysis than Amberlyst-15 resin (75).

The sulfonated surface groups are occasionally leached during hydrolysis reactions, however, the deactivation of these sulfonated carbon does not happen at low temperature (90ºC) (68).

1.2.4.1.1.4 Functionalized silica

Silica is a group of catalysts with an extensive range of porosities and surface areas, from mesoporous materials like amorphous silica to microporous materials like SBA-15 or MCM-41. Their intrinsic acidity is not high but they can be improved by treatments including sulfone or sultone compounds and then aggregate sulfonic (SO_3H) groups. As already was mentioned before in the case of carbon materials, functionalized silica can also have hydroxyl or carboxylic groups. Reports are suggesting that sulfonic groups release protons in the aqueous medium to hydrolyze cellobiose (69). Following this idea, the reactant is not in close contact with the solid catalyst and the diffusion rate does not limit the reaction kinetics. Several authors also reported good stability of sulfonated silica during disaccharides hydrolysis.

Even if the intrinsic nature of heterogenous catalysts already allows an easy separation, there is a way to improve the catalyst recovery by adding paramagnetic iron species like already was tested on SBA-15-SO_3 (76). This procedure was also applied to nanoparticles $CoFe_2O_4$-SiO_2-SO_3H for cellobiose hydrolysis, with glucose yields reaching 50% for an 80% cellobiose conversion (68).

1.2.4.1.1.5 Heteropoly acids

Heteropoly acids (HPAs) are protonic acids that incorporate polyoxometalate anions having metal-oxygen octahedral as the basic structural units into the complex cluster. Micellar heteropoly acids facilitate the interactions of acid site-reactant, proving to be efficient in the hydrolysis of sucrose.

Additionally, these catalysts present some advantages like being stable in the reaction medium, the capacity to hydrolyze cellobiose into glucose at relatively low temperatures (150ºC), and their super acidity. Some HPAs exhibit an acid capacity even stronger than H_2SO_4. The main documented drawback is their solubility in polar solvents, but they can be supported on silica, carbon, or acidic ion-exchange resins (68).

Heteropoly acids (HPA) in combination with supported Ru catalysts (Ru/C) allow conversion of cellulose with above 80% yield of C4–C6 sugar alcohols and 91% carbon efficiency at 160ºC, but may even be applied effectively in the transformation of spruce as a real biomass feedstock (77).

Table 1.3 show some literature results of heterogenous acids.

Table 1.3- Summary of several heterogeneous acid catalysts found in literature, in terms of operation conditions and performance results in glucose-based disaccharides hydrolysis.

Catalyst	***Type***	***Reactant***	***Conditions***	***Catalyst/Substrate (w/w)***	***Conversion (%)***	***Sugar yield (%)***	***Ref***
H-Y (Si/Al=27)	Zeolite	Sucrose	70ºC, 8h	0.1	<5	-	(78)
H-Y (Si/Al=15)	Zeolite	Sucrose	95ºC, 3h	0.01	100	-	(78)
H-ZSM5	Zeolite	Cellobiose	95ºC, 1h	-	-	0	(79)
H-MOR	Zeolite	Maltose	130ºC,24h,	0.5	60-70	66	(80)
Amberlite IR120 H	Resin	Sucrose	170ºC,1h	2.6	85-100	-	(81)
Amberlite IR120 H	Resin	Sucrose	79ºC, 1.5h,	3.6	100	-	(82)
Amberlite 200C	Resin	Sucrose	80ºC,3h	1.6	100	98	(83)
Nafion NR50	Resin	Cellobiose	100ºC,4h	2	-	6.7	(84)
Amberlyst-15	Resin	Cellobiose	100ºC,4h	2	-	7	(84)
Amberlyst-15	Resin	Cellobiose	100ºC,2h	0.2	-	87	(85)
Nafion NR50	Resin	Cellobiose	100ºC,4h	0.2	-	75	(85)
Carbon-SO_3	Carbon material	Cellobiose	90ºC,24h	0.8	85	85	(86)
Biochar-SO_3H	Carbon material	Cellobiose	123ºC,7h	10	97	80	(75)
SBA-15-COOH	Functionalized silica	Sucrose	80ºC,4h	0.5	68	-	(87)
MCM-41	Functionalized silica	Maltose	130ºC,24h	0.5	74	-	(80)
$H_4SiW_{10}O_{40}$	Heteropoly acid	Cellobiose	150ºC,24h	1	61	53	(88)
$H_3PMo_{12}O_{40}$	Heteropoly acid	Cellobiose	150ºC,24h	1	83	48	(88)

1.2.5 Hemicellulose valorization products

To achieve the valorization of the hemicellulosic fraction of the biomass, dilute acid hydrolysis and hydrothermal treatments are among the most appropriate fractionation methods as they are very selective for this fraction. Depending on the severity of the treatment, the products resulting from this solubilization are oligosaccharides (OS), monosaccharides, products resulting from the degradation of monosaccharides, and acetic acid. At moderate conditions, OS is, in general, the main product obtained, with the relative proportions of OS/monosaccharides/degradation products varying according to operational conditions and the type of pre-treatment. Among the various hydrothermal treatments, autohydrolysis generally allows higher OS yields, in contrast to the steam explosion, where monosaccharide yields are higher. This is, in fact, a typical characteristic that distinguishes these two types of treatments.

Oligosaccharides (OS) are low or medium molecular weight compounds that have monosaccharides linked by glycosidic bonds. Some authors consider that OSs generally contain between 3 and 10 units of monosaccharides (89), although OSs with a higher degree of polymerization is often included (90).

The hydrolytic processes to produce OS have been mainly applied to hardwoods and agriculture by-products, which hemicelluloses are mainly composed by xylan, and consequently producing xylo-oligosaccharides (XOS). XOS are OS composed of linear xylose chains and may contain residues of arabinose or other “oses”, acetyl groups, and uronic acids as side substituents. Some of these XOS has been referred to as prebiotic products that have similar or even improved properties compared to other prebiotics already recognized, such as fructo-oligosaccharides (FOS) (91-93).

Commercial XOS is obtained by the enzymatic treatment of materials rich in xylans, characterized by having a low degree of polymerization (mainly consisting of xylobiosis and xylotriosis). An economical alternative for obtaining XOS with different characteristics, consists of selective fractionation of lignocellulosic materials by hydrothermal processes, as is the case with autohydrolysis. To obtain compounds with a lower degree of polymerization, several approaches can be used. The direct enzymatic hydrolysis of the raw material can be an option, or a chemical treatment can be carried out to fractionate the ML to isolate (or solubilize) the xylans, followed by enzymatic hydrolysis of the polymers to XOS. The hydrolytic degradation of xylans to XOS can also be promoted using aqueous processes (water or steam) or dilute acid solutions.

The production of XOS, by combining chemical and enzymatic processes, consists of obtaining soluble xylan fragments, for example, through alkaline extraction, diluted acid hydrolysis, or hydrothermal treatments. Of these, the most used process is alkaline extraction, although the high cost of reagents and hydrolysis times, associated with the need for further neutralization, limit their application at an industrial level (94). Diluted acid hydrolysis has the disadvantage of leading to the formation of a high number of monosaccharides and degradation products.

1.2.6 Pre-treatment severity

The determination of the optimal operating conditions of the hydrolysis treatment for a given lignocellulosic material is achieved by establishing the relationship between the process variables and

the chemical changes that occur in the substrate. One way of analyzing pre-treatments is through severity parameters, which seek to combine the effect of different operational variables into a single empirical parameter.

1.2.6.1 Severity factor

The severity factor relationship developed by Chornet and Overend (95) ponder temperature against time to predict conditions that result in similar yields from the cleavage of hemicellulose to its component sugars according to the following relationship in which R_o is the severity factor, t is the time in minutes, and T is the temperature in ºC. The exponential term is consistent with the empirical that the reaction rate will double for every 10 ºC increase in temperature. Therefore, Eq. (1) predicts that the value of R_o will remain the same if the time t is cut in half for every 10ºC increase in temperature. Additionally, once a combination of time and temperature has been identified to give a target yield, Eq. (1.1) can be used to project the approximated time needed to carry out the reaction for a different temperature (96).

$$R_o = \int_0^t exp\left(\frac{T-100}{14.75}\right) dt \qquad \text{Equation 1.1}$$

To combine the effects of temperature, time, and acid concentration on hemicellulose release from biomass and facilitate the study of the cooperation between these three operational parameters, Chum et al. and Abatzoglou et al. developed the combined severity factor (97, 98).

Taking advantage of the definition of pH, log CS can be represented by the next equation.

$$Log\ CS = logR_o - pH \qquad \text{Equation 1.2}$$

1.2.6.2 Modified combined severity factor

Within the scope of this work, a modified combined severity was developed where this factor is the sum of a portion that represents the temperature and reaction time (log Ro) and a portion that represents the acidity used (log [H_2SO_4]).

$$mCS = logR_o + \log\,[H_2SO_4] \qquad \text{Equation 1.3}$$

In this new approach, we are not directly dependent on the concept of pH where lower value represents greater acidity, but rather on the molar amount (mmol) of the acid present in the process. The concentration unit must be millimolar to avoid negative numbers when the logarithmic function is applied. Representing the combined severity in this way turns evident the fact that greater acidity will reflect greater severity.

1.3 Objectives

The reduction of operating costs related to the recovery of hemicellulosic sugars is still one of the critical points in the development of biorefineries, and this work aims to contribute to improve this by exploring the utilization of ion exchange resins as solid acid catalysts.

The main goals are the development and evaluation of the effect of solid acid catalysts on the recovery of hemicellulosic sugars from raw biomass and the hydrolysis of hemicellulose derived oligosaccharides as a way to contribute to the extensive research in the field of the biorefineries and the bioeconomy.

The focus of the study in on the effect of process conditions, including temperature, reaction time, acidity, and solid catalyst characteristics on the performance of deconstruction of three different types of feedstock. The research was conducted both on raw biomass, and on liquors/hydrolysates from future standard industrial pre-treatments such as autohydrolysis and organosolv. The evaluation of such performance was based on treatment effectiveness for the removal of hemicellulose as determined by the characterization of resulting liquid fractions of pretreated biomass.

The effect of solid acid catalysts is explored in more detail for batch operation, but flow-through operation is also tested.

2 Material and methods

2.1 Feedstock and commercial oligosaccharides

The wheat straw (WS) sample and Miscanthus pellets (8.9 mm dia. x 13 mm length) were kindly provided by ECN-TNO (Netherlands). The Eucalyptus residues (ER) were kindly provided by The Navigator Company from their paper mill in Cacia, Portugal. ER and WS were stored at room temperature and used without any milling in autohydrolysis, organosolv, and homogenous diluted acid hydrolysis. For heterogeneous acid hydrolysis, these feedstocks were grounded with a knife mill IKA® WERKE, MF 10 basic (Germany) equipped with a 0.5 mm screen, homogenized in a defined lot, and stored in plastic containers at room temperature, before use. Miscanthus was always used in pelletized form.

The commercial oligosaccharide used was -α-D-Galactopyranosyl-(1→6)-α-D-glucopyranosyl β-D-fructofuranoside (D-(+)-Raffinose pentahydrate) from Merck Millipore, Germany.

2.2 Catalysts and other reagents

In terms of reagents, the following were used: distilled water produced by the PURELAB Classic Elga system (ELGA LabWater, UK), Baysilone oil M300 (Bayer, Germany), Sulfuric acid (96 wt.%, Panreac, Spain) was used as catalysts and sodium hydroxide (eka, Portugal) was used for the quantification of Brönsted acid sites. D-Glucose (≥98 wt.%, Merck, Germany), D-Xylose (≥98 wt.%, Merck, Germany), L-Arabinose (≥ 98 wt.%, Merck, Germany), Furfural (99 wt.%, Sigma-Aldrich, Germany), 5-Hydroxymethylfurfural (99 wt.%, Sigma-Aldrich, Germany), Formic acid (98%, Panreac, Spain), and Acetic acid (glacial, 99.8 wt.% Merck, Germany) were used as standards for HPLC analysis.

Table 2.1 shows the solid acid Ion Exchange Resins used in this work and their main physical-chemical characteristics.

2.3 Selective fractionation of Eucalyptus residues, Wheat straw, and Miscanthus

2.3.1 Autohydrolysis process

Experiments were carried out using a two-liter reactor (Parr Instruments Company, Moline, IL, USA) heated externally. Two turbines, of four blades each, agitated the mixture. Tap water passing through an internal serpentine was used to cool the system, when necessary. Temperature and agitation were monitored and controlled with a 4842 PID controller (Parr Instruments Company). The agitation was set to 150 rpm. The pressure was measured by the same controller.

Table 2.1 Physic-chemical properties/characteristics of the IERs used in this research work.

Resin	*Physical Form*	*Particle Size Uniformity coefficient (mm)*	*Matrix Type*	*Matrix Composition*	*Functional Group*	*Max Temp (ºC)*	*Ionic Form as Shipped*	*Moisture holding capacity (%)*	*Total Exchange Capacity (eq/L)*	*Brand*
Amberlite IR120	Amber spherical beads	≤ 1.8	Gel	Styrene divinylbenzene crosslink	Sulfonic acid	135	H^+	53 to 58	≥ 1.80	Sigma-Aldrich
Amberlyst 15	Gray, opaque, spherical beads	<0.3	Macroporous	Styrene divinylbenzene crosslink	Sulfonic acid	120	H+	52 to 57	1.7	Sigma-Aldrich
Amberlite IRC748	Opaque, beige beads	0.50 - 0.65	Macroporous	Styrene divinylbenzene crosslink	Iminodiacetic acid	90	Na^+	60 to 65	≥ 1.35	Rohm and Haas
Amberlite IRA200	Gray spherical beads	≤ 1.7	Macroporous	Styrene divinylbenzene crosslink	Sulfonic acid	135	Na^+	46 to 52	≥1.7	Alfa Aesar
NAFION NR50	Colorless to Light Brown	1.7	-	Perfluorinated ether vinyl copolymer(99)	Sulfonic acid	200	H^+	-	≥0.8	Sigma-Aldrich

Water was added to biomass to reach a liquid-to-solid ratio (LSR) of 7 (g water/g dry biomass) and a total mass of 1500 g. The treatments are conducted under non-isothermal conditions, heating up to 190ºC, followed by rapid cooling to 100ºC. The reactor was then removed from the heating jacket and the vessel was left at room temperature and only then open. Solid and liquid phases were separated by pressing (up to 200 bar) with a manual hydraulic press (Sotel, Portugal). After pressing, the liquor obtained was filtered under vacuum (Quantitative filter paper, 20-25 µm, Filter-Lab) and stored at 4ºC for further compositional analysis.

2.3.2 Ethanol-based organosolv process

Organosolv treatments were similar to the autohydrolysis treatment procedures described above with the following variations: the LSR used was 10, and instead of water, ethanol 50% (w/w) was added to biomass. The temperature tested was also 190ºC, but the reaction was held at isothermal conditions for 2 h. Upon cooling to room temperature, the solid phase was separated by pressing, as above, and then washed with 2 L of ethanol 50% (w/w).

2.3.3 Optimization of the dilute acid hydrolysis with sulfuric acid

The optimization was carried out by testing different concentrations of H_2SO_4 and reaction times according to a Doehlert experimental design matrix (Table 2.2 and Table 2.3)., resulting in 7 combinations. The Doehlert matrix was used to establish the effects of the concentration of H_2SO_4 (X_1) between 0 and 4% (w / w) and reaction time (X_2) between 0 and 120 min in a LSR of 5 $g.g^{-1}$ (between 5 g of the acid solution and 1 g of biomass) for Miscanthus . For ER the acid concentration varied between 0 and 2% (w / w) and the reaction time between 0 and 240 min in an LSR of 6.5 $g.g^{-1}$. Both were performed at 130 ºC. Within these ranges, 5 concentrations of H_2SO_4 and 3 operating times were chosen, which allowed the estimation of the curvature effects for each independent variable.

Table 2.2- Coded matrix for Doehlert experimental design for two experimental variables and the corresponding experimental matrix for Miscanthus.

Essays	Variables			
	Coded		Real	
	X_1	X_2	H_2SO_4 (%)	Time (min)
A	0,000	0,000	2.00	60.00
B	1,000	0,000	4.00	60.00
C	-1,000	0,000	0.00	60.00
D	0,500	0,866	3.00	111.96
E	-0,500	-0,866	1.00	8.04
F	0,500	-0,866	3.00	8.04
G	-0,500	0,866	1.00	111.96

Table 2.3- Coded matrix for Doehlert experimental design for two experimental variables and the corresponding experimental matrix for Eucalyptus.

Essays	Variables			
	Coded		Real	
	X_1	X_2	H_2SO_4 (%)	Time (min)
A1	0,000	0,000	1.00	120.00
B1	1,000	0,000	2.00	120.00
C1	-1,000	0,000	0.00	120.00
D1	0,500	0,866	1.50	223.92
E1	-0,500	-0,866	0.50	16.08
F1	0,500	-0,866	1.50	16.08
G1	-0,500	0,866	0.50	223.92

The biomass was hydrolyzed in pressure tubes (25 mL microreactors) with Teflon screw caps (ACE Glass Inc., USA). The microreactors were placed in a silicone-oil bath previously preheated to the chosen temperature.

Homogenization was assured by magnetic stirring, both of the oil bath and in the microreactors. Once the reaction was finished, the reactors were removed from the bath and allowed to naturally cool down to room temperature. Aliquots of the recovered liquid hydrolysates were filtered through 0.45μm nylon filters and analyzed by HPLC and for soluble phenolic content via UV spectrophotometry. All samples had their pH characterized and all experimental tests were performed in duplicate.

The model used to express the responses was a second-order polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \qquad \text{Eq. 2.1}$$

where Y is the answer variable, X is the independent variable, which means that X_1 refers to the concentration of H_2SO_4 and X_2 to the reaction time. B_0 is the regression coefficient at the origin; β_1 and β_2 are the linear coefficients of variables 1 and 2, respectively; β_{12} is the second-order interaction coefficient between variables 1 and 2; β_{11} and β_{22} are the quadratic coefficients for variables 1 and 2, and ε are the independent random errors.

Multiple linear regression to Eq. 2.1 and analysis of variance (ANOVA) were performed using the Microsoft® Excel 365 regression toolset, for all replicates. The best hydrolysis conditions were determined based on the best fit equations, using the Microsoft® Excel 365 “solver” tools The applied algorithm is GRG non-linear with restriction between -1 and 1.

The coded representation of the variables was used for all calculation purposes.

2.3.4 Optimization of the dilute acid hydrolysis over Ion Exchange Resins

The dilute acid hydrolysis with Ion Exchange Resins (IERs) experiments was carried out, in batch operation, in pressure tubes (25 mL) with Teflon screw caps (ACE Glass Inc., USA), using 0.3 g of biomass, 0.1g of IER and 4.6g of distilled water, representing 6% of solid loading($g_{feedstock}/g_{total}$) and 2% of catalyst ($g_{catalyst}/g_{total}$). The tubes were placed in a silicone-oil bath previously preheated to the

chosen temperature. Homogenization was assured by magnetic stirring. Bath temperature was kept constant at 140 (±1ºC) utilizing a temperature controller (IKA C-MAG HS7). Pre-treatments were carried out between 30 and 120 minutes with different kinds of feedstock and IERs. Once the reaction was finished, the contents of the pressure tubes were allowed to naturally cool down to room temperature. Aliquots of the recovered liquid hydrolysates were filtered through 0.45μm nylon filters and analyzed by HPLC and for soluble phenolic content via UV spectrophotometry.

2.4 Hydrolysis of oligosaccharides over ion exchange resins

2.4.1 Catalyst development

2.4.1.1 Production of catalytic activated carbons

The preparation of activated carbons from sucrose and biomass (pelletized Miscanthus) was carried out in four stages: (i) Sucrose/biomass was treated with commercial sulfuric acid (96 wt.%, Panreac, Spain). (ii) The acidified biomass was allowed to react for 24 h without agitation at 60ºC. (iii) After the reaction, the sample was washed several times with distilled water to remove residual chemicals. (iv) The washed sample was dried at 60ºC for 24 h to prepare the activated carbon.

2.4.1.1.1 The magnetization of activated carbons

Magnetized activated carbon was prepared from the previously prepared activated carbon based to the procedure described in (100). The activated carbon (5 g) was suspended in distilled water (50 mL). An aqueous 1.4% ferric chloride solution was freshly prepared. An aqueous 13.3% ferrous sulfate solution was prepared. Both solutions were combined and vigorously stirred at 60–70 ◦C. The suspension formed was then added, at room temperature, to the activated carbon aqueous suspension and slowly stirred for 30 min. After mixing, 10 M NaOH aqueous solution was added dropwise into the suspension until the pH raised to 10–11. During NaOH addition, the suspension became dark brown at pH ∼ 6 and then black at pH ∼ 10. After mixing for 60 min, the suspension was aged at room temperature for 24 h and then repeatedly washed with distilled water. The obtained materials were vacuum filtered and dried overnight at 50 ◦C in a hot air oven.

2.4.1.2 Ion Exchange Resins activation

The activation of IERs was performed adding H_2SO_4 (4% w/w) solution to the catalyst in a proportion 1:1 (w/w) and incubating for 30 minutes under magnetic stirring, after which, the acid solution is decanted and then washed using distilled water in a proportion of 5:1 (water/catalyst, w/w) and incubated for 5 minutes under magnetic stirring. After decanting, the retaining catalysts were dried in an oven (45ºC) for 24h.

2.4.2 Process development

2.4.2.1 Hydrolysis of commercial and hemicellulose-derived oligosaccharides

The hydrolysis of commercial oligosaccharides experiments were carried out in pressure tubes (25 mL) with Teflon screw caps (ACE Glass Inc., USA), using a solid acid to commercial oligosaccharides ratio of 5%. The tubes were placed in a silicone-oil bath previously preheated to the prescribed temperature 140 ºC (±1ºC). The hydrolysis was carried out for 1h with different solid acid catalysts. Once the reaction was finished, the contents of the pressure tubes were allowed to naturally cool down to room temperature. Aliquots of the recovered liquid hydrolysates were filtered through 0.45μm nylon filters and analyzed by HPLC and for soluble phenolic content via UV spectrophotometry. The experiments were conducted at least in duplicate.

Hemicellulose-derived oligosaccharides testing was carried out as described above.

2.4.2.2 OS Hydrolysis: Catalyst reutilization

The catalyst reutilization is applied for, both, the hydrolysis of commercial oligosaccharides and hemicellulose-derives oligosaccharides. This reutilization was carried out through decantation of the hydrolyzed liquor after each experiment, being repeated as long as there is no decrease in the efficiency of the catalysts used.

2.4.2.3 Flow-through OS Hydrolysis

Flow-through pre-treatment experiments were carried out in a Dionex™ ASE™ 150 Accelerated Solvent Extractor (Figure 2-1) from ThermoFisher Scientific, USA. A weighted amount of IERs (1.5 g) was mixed with solid-glass beads for support and loaded into a 5 mL stainless steel cell. A layer of solid-glass beads and glass fiber filters (1.2 μm, VWR) was placed at the top and bottom of the cell to prevent clogging of the cell seals. The cell was placed into the oven of the ASE system, previously pre-heated to the required hydrolysis temperature, in a vertical position. The commercial sugar raffinose was placed in the pressure-resistant glass solvent bottle which was admitted to the extraction cell in a vertical top to bottom flow configuration. The system performed a series of 5 cycles, each consisting of 1) quick filling of the extraction cell by a 0.4 mL/min pump, 2) followed by a static period of several minutes (5 min) while the catalyst and the liquor were kept at constant hydrolysis temperature and around 100 bar, 3) after which the cell was decompressed and the liquid released to the collection 250 mL glass bottle (previously weighted for determination of liquor recovery).

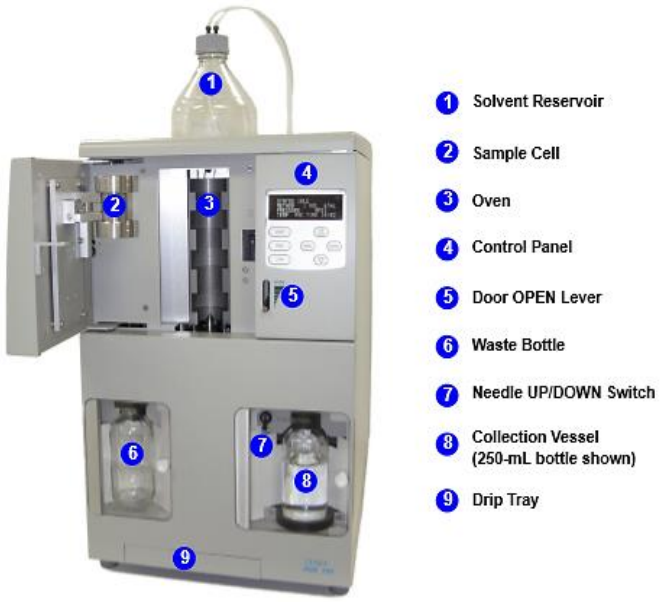

Figure 2-1. Dionex™ ASE™ 150 system used for flow-through OS Hydrolysis

After the completion of the hydrolysis, the extraction cell and collection bottle were left at room temperature to naturally cool down. The extraction of cell content was kept inside for further repetitions of the process. The recovered liquor was stored at 4ºC for posterior filtration through 0.45μm nylon filters and HPLC and UV spectrophotometry analysis.

Table 2.4. Flow-through operation conditions during OS Hydrolysis

	Flow-through
Temperature (ºC)	140
Pressure (bar)	80-120
Catalyst/run (g/ml)	0.15

2.5 Analytical methods

A detailed explanation of performed calculations and the used equations associated to the methods described below can be seen in Supplementary material: Annex 1: Mathematical formulae.

2.5.1 Feedstock characterization

2.5.1.1 Physical characterization - Granulometric characterization

Particle size characterization was performed using a test sieve shaker (EVS1, Endecotts, England) and six sieves (ASTM E11, Retsch, Germany) with different pore sizes arranged in crescent series according to the pore diameter: 0.25, 0.50, 1.00, 1.60, 2.36 and 3.55 mm. To carry out the analysis, 3 samples (approximately 100 g each), were screened for 20 min, after which the pre-weighed sieves containing the biomass were weighed in a precision balance (N2B110 Navigator, OHAUS, Switzerland).

2.5.1.2 Determination of moisture and ash content

The moisture content of solid samples was determined based to the NREL's laboratory analytical procedure (LAP) NREL/TP-510-42621(101). Porcelain crucibles were oven-dried at 100ºC for at least 12 hours, then cooled down in a desiccator to room temperature and weighed on an analytical balance (Mettler 160 HK, Switzerland) to the nearest 0.1 mg. Thereafter, 1 g of sample was weighed to the nearest 0.1 mg in the previously weighed crucibles, which were then placed in an oven at 100ºC for a minimum of 12 h. The crucibles containing the dried samples were then cooled down in a desiccator and reweighed.

Ash content was determined according to NREL/TP-510-42622 protocol (102). Porcelain crucibles were dried to constant weight in a muffle furnace at 550ºC for at least 5 h and weighed to the nearest 0.1 mg on an analytical balance (Mettler 160 HK, Switzerland), after cooling in a desiccator. The solid samples (0.5±0.0001 g) were loaded into the tared crucibles, burned with a heating plate and then placed in the muffle at 550ºC for at least 18 h. The crucibles with the remaining ash were cooled to room temperature in a desiccator and then weighed. Ash content was determined calculating the difference between the final weight of the crucibles and its tare.

The determination of the moisture and ash content samples was performed in duplicate and reported as mean values.

2.5.1.3 Determination of structural carbohydrates, acetyl groups, and Klason lignin

Original and pretreated solids were subjected to a process of quantitative acid hydrolysis following NREL/TP-510-42618 protocol (103). Solid samples placed into test tubes were rigorously weighed (200.0 ± 10.0 mg) to the nearest 0.1 mg on an analytical balance (Mettler 160 HK, Switzerland). Sulfuric acid 72% w/w (2 mL) was added to test tubes containing the solid sample, which were kept for 1 h at 30ºC in a Memmert (Schwabach, Germany) W350 water bath, manually stirred every 10 minutes with a glass stirring rod, to ensure proper contact between the samples and the acid enabling complete

dissolution of the polysaccharides. The content of the tubes was then transferred to 250 mL Duran™ Schott™ borosilicate glass flasks with screw caps, diluted with distilled water to a concentration of 4% (w/w) of H_2SO_4 and placed inside an autoclave (Uniclave, Portugal) at 121ºC for 1 h, to ensure complete hydrolysis of sugars to monosaccharides and acetyl groups to acetic acid. After cooling down, the mixture was filtered through previously dried (in a muffle furnace) and weighted sintered glass crucibles (#3 porosity). The solid remaining in the crucibles (corresponding to Klason lignin and ash) was washed with 100 mL of distilled water and dried in an oven at 105ºC to constant weight and then burned in a muffle furnace for gravimetric determination of ash. An aliquot of the obtained liquid phase was filtered through 0.45 μm nylon filters and analyzed via HPLC for monomeric sugars and acetic acid. A multiplying factor of 1.04 was considered for hexoses and 1.09 for pentoses, for taking into account sugar degradation during the hydrolysis step. Mass concentrations of monomeric sugars were converted into polysaccharides and acetyl groups concentrations considering the respective factor(Annex 1: Mathematical formulae), which accounts for the gain of a water molecule per each monomer molecule during acid hydrolysis of the polymers. For each sample, the procedure was performed in duplicate, and results were reported as mean values on a dry basis.

2.5.1.4 Quantification of soluble phenolics and acid-soluble lignin

Pre-treatment liquors and hydrolysates from quantitative acid hydrolysis were analyzed for soluble lignin/phenolic content following NREL/TP-510-42618 protocol (103). Background of the same solvent of the analyzed sample was run in a Jasco 7800 UV-vis spectrophotometer (Jasco Inc., Japan). The absorbances of the liquid samples were measured at 320 nm as recommended (104), after dilution with distilled water as appropriate to obtain absorbance values below 1.0. Absorbance values were then converted to phenolic content through the Lambert-Beer Law, considering an extinction coefficient of 30 $Lg^{-1}cm^{-1}$ for ER (103) and 16 $Lg^{-1}cm^{-1}$ for WS (105), while accounting for the performed dilution. Each sample was analyzed in triplicate and the average values reported.

2.5.2 Hydrolysates characterization

2.5.2.1 pH

The pH measurements were carried out using a pre-calibrated pH electrode linked to a chemtrix type 45AR pH controller (Netherlands). The controller was calibrated with 4.00 and 7.00 pH value buffers. The pH electrode was thoroughly rinsed between measurements with distilled water to prevent carryover contamination of the tested solutions.

2.5.2.2 Quantification of monosaccharides, aliphatic acids, and furans by HPLC

Quantification of sugars (glucose, xylose, and arabinose), aliphatic acids (acetic and formic acids) and furans (HMF and furfural) in the liquors obtained from the pre-treatments, the hydrolysates from quantitative acid hydrolysis and the enzymatic digests was performed using an Agilent 1100 Series HPLC system (Waldbronn, Germany), equipped with an Aminex HPX-87H column (Bio-Rad, Hercules,

CA, USA), with a 7.8 mm diameter and 300 mm length. Analyses were performed at 50ºC, using 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min, with an injection volume of 5 µL. All samples were filtered using nylon filters with a pore size of 0.45 µm before being injected in the HPLC system. The detection was performed using a RID (refractive index) detector at 50ºC for monosaccharides (glucose, xylose, and arabinose) and aliphatic acid quantification, and a DAD UV/Vis photodiode detector set at 280 nm for furans (furfural and 5-hydroxymethylfurfural)(Table 2.5). The quantification was performed by external calibration using standard solutions of the measured compounds (HPLC grade). The calibration curves were plotted as the area of the compound peak obtained in the respective chromatogram versus the compound concentration, varying between 0.01-0.1 g/L and 0.5-20 g/L. The appropriate range selected for quantification was always the one that included the concentration to be determined. The analysis of the obtained chromatograms, including the identification of compound peaks (by their elution time) and their integration (area determination for quantification) was performed using Agilent™ ChemStation™ for LC 3D systems (Agilent Technologies, 2001-2005, USA).

Table 2.5 Characteristics and operational conditions of the chromatographic column HPX-87H.

Characteristics	**Operational conditions**
Column dimensions	300 × 7.8 mm
Mobile phase	H_2SO_4 5 mM
Flow	0.6 mL/min
Injection volume	5 µL
Column temperature	50ºC
IV detector temperature	50ºC
UV wavelength	280 nm

2.5.2.3 Quantification of oligosaccharides

The oligosaccharides (OS) were measured by an indirect method based on quantitative acid hydrolysis according to NREL/TP-510-42623 (106)

Concentrated sulphuric acid (72% (w/w)) was added to an aliquot of liquor resulting from the treatments, to reach a final H_2SO_4 concentration of 4% (w/w). The mixture was placed in an autoclave and hydrolyzed at 121ºC for 1 h. After completion of the autoclave cycle, the hydrolysates were slowly cooled down to room temperature. After cooling, a sample was collected, filtered using 0.45 µm membranes (Millipore®), and analyzed by HPLC The OS concentrations were calculated from the increase in sugar monomers. This procedure was always performed in duplicate (for calculations see Annex 1: Mathematical formulae).

2.5.3 Solid acids characterization

2.5.3.1 Quantification of the Brönsted acid sites

The quantification of the number of Brönsted-acid sites was carried out based on the method described in (107). A NaOH solution (20.0 mL) with a concentration of 0.01M was added to 0.04 g of solid catalyst to be characterized and left to react for 2 hours under magnetic stirring at room temperature in a capped flask. This procedure was carried out in triplicate. Thereafter, an 8 ml aliquot from each of the previous solutions was sampled, taking care not to remove the solid catalyst, into an Erlenmeyer flask of at least 50 mL. These aliquots were titrated with 0.01 M HCl using phenolphthalein as the indicator, at least in duplicate

3 Results and discussion

3.1 Feedstock characterization

To get a deeper knowledge about the raw material used in this work, as well as to understand its behavior during the pre-treatments, its physical and chemical characterization was performed. These characterizations allow the understanding of the material in future steps as well as its comparison with other materials.

3.1.1 Physical characterization

3.1.1.1 Particle size distribution

A particle size characterization was carried out to ER and WS to determine the percentage of the fractions, between 0 mm and 3.55 mm (Figure 3-1).

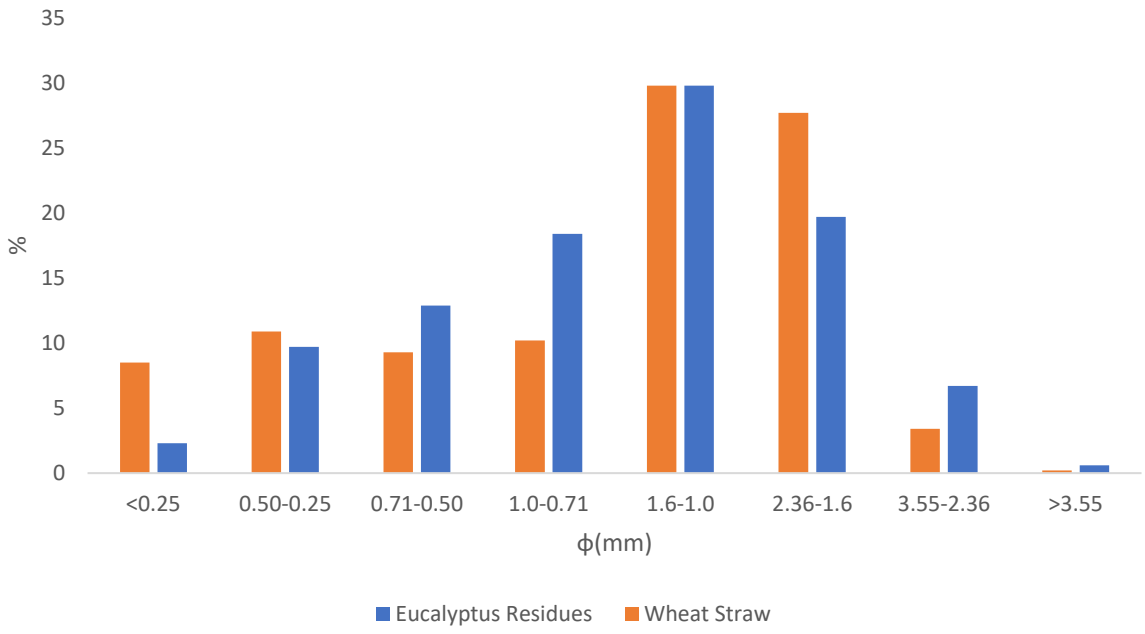

Figure 3-1- Particle size distribution for ER and WS.

Fractions with particle size ranging between 1.0 and 1.6 mm correspond to 28.8% of the total mass for both types of feedstock, being the most representative fraction followed by 1.6 to 2.36 mm fraction corresponding to 27.7% and 19.7% for WS and ER, respectively. The fraction corresponding to fine particles (< 0.25 mm) represents only 2.3% of total mass for ER and 8.5% for WS. The largest particles, >3.55 correspond only to 0.2% and 0.6 of total WS and ER analyzed, respectively. The mean diameter of ER and WS is 1.6 mm and 1 mm, respectively

As no fraction exceeds 50% of the total, it was decided to use all the fractions in the subsequent treatments.

3.1.2 Chemical characterization

The structural composition (in the percentage of dry weight) of feedstocks used in this work was determined by quantitative acid hydrolysis (Figure 3-2). The results are in line with previous reports (108) that assure its suitability for upgrade in the biorefinery framework. The first division that can be made here is between hardwoods and grasses, where ER belongs to the former and WS and Miscanthus to the latter (109). ER has higher glucan content and less xylan plus arabinan (hemicellulose) content than WS and Miscanthus. These two latter feedstocks present similar glucan and hemicellulose content, however, WS has higher ash content and fewer acetyl groups and lignin than Miscanthus. ER is the feedstock with higher acetyl group percentage, being second to Miscanthus in lignin.

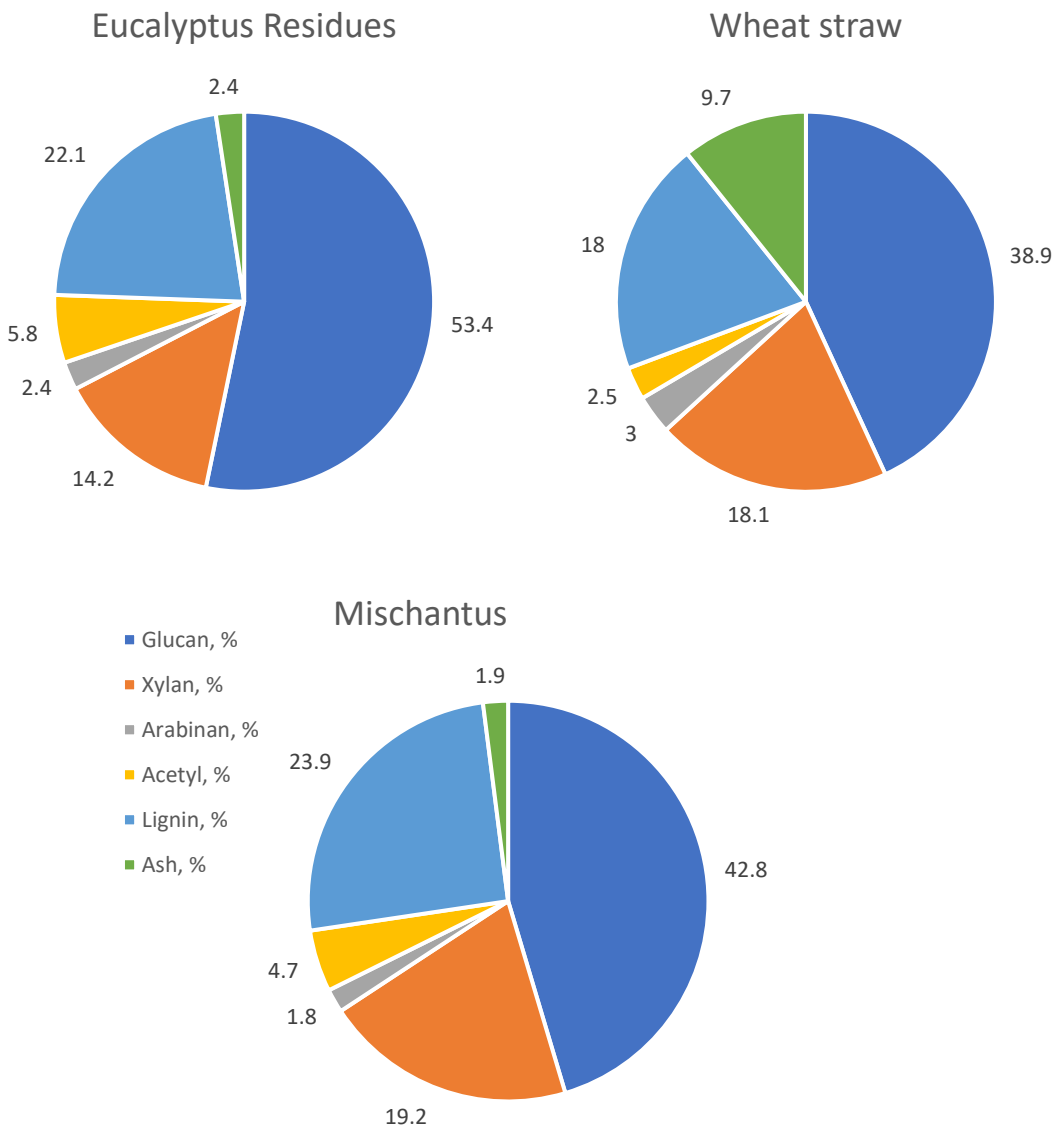

Figure 3-2-Structural composition of ER, WS, and Miscanthus (%, dry weight)

3.2 Catalyst selection

3.2.1 Solid acid catalyst characterization

Realizing that the H^+ form is essential to acidify the medium and that increasing the amount of H^+ ions reproduced changes in the resins hydrolysis efficiencies, the Brönsted Acid sites (BAS) were quantified (amount of protons H^+ each g of resin can donate) to characterize the hydrolysis capacity of each resin and represented in Figure 3-3.

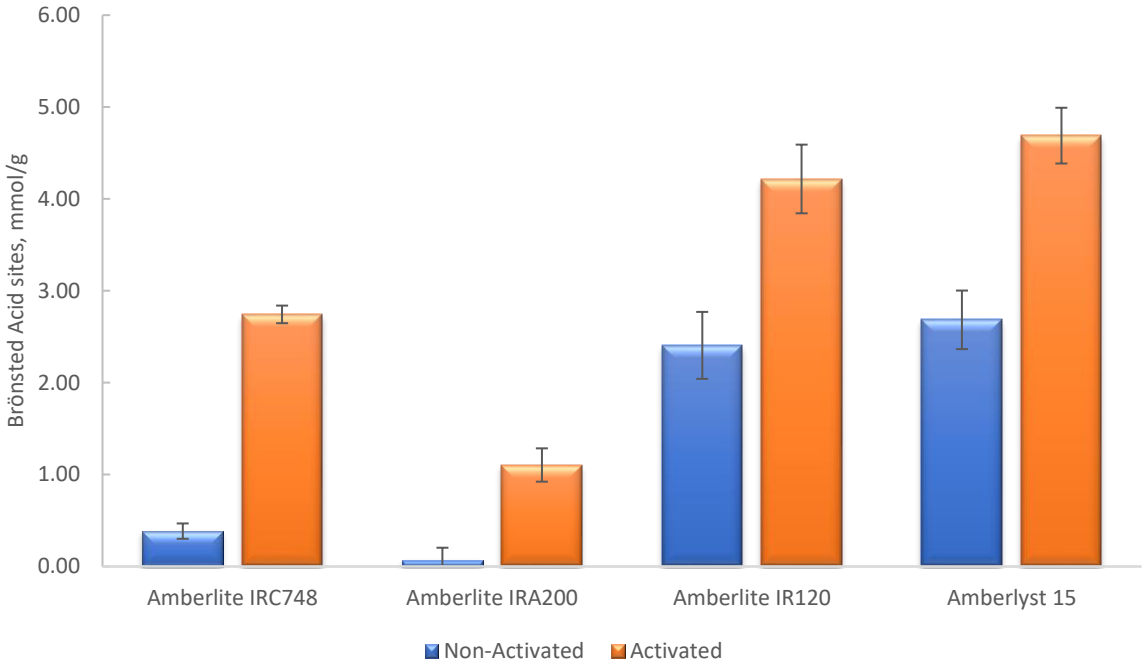

Figure 3-3- BAS of the different IERs tested

The data show a huge difference between the BAS present in the non-activated resins of Amberlite IR120 and Amberlyst 15, which have 2.41 $mmol.g^{-1}$ and 2.68 $mmol.g^{-1}$, respectively, and Amberlite IRC748 and IRA200, which have 0.38 $mmol.g^{-1}$ and 0.07 $mmol.g^{-1}$, respectively. The low-efficiency values in these last two resins are due to the almost zero amount of H^+ protons linked to their functional groups, although Amberlite IRC748, in addition to the higher ion exchange speed mentioned above, has a value of BAS slightly higher which explain the better efficiency of this resin concerning Amberlite IRA200. By activating the resins, all the catalysts increased their number of H^+ protons. Amberlite IRC748 increased 2.36 $mmol.g^{-1}$, Amberlite IRA200 increased 1.03 $mmol.g^{-1}$, Amberlite IR120 increased by 1.81 $mmol.g^{-1}$ and Amberlyst 15 increased by 2.01 $mmol.g^{-1}$. According to the literature, an hydrolysis catalyst must bear strong Brønsted acid sites (68).

3.2.2 Raffinose hydrolysis

The standard OS used to test different IERs was commercial raffinose. Raffinose is a trisaccharide in which glucose acts as a monosaccharide bridge between galactose and fructose (Figure 3-4). It has both α and β glycosidic bonds and can, therefore, be hydrolyzed to D-galactose and sucrose or to melibiose and D-fructose.

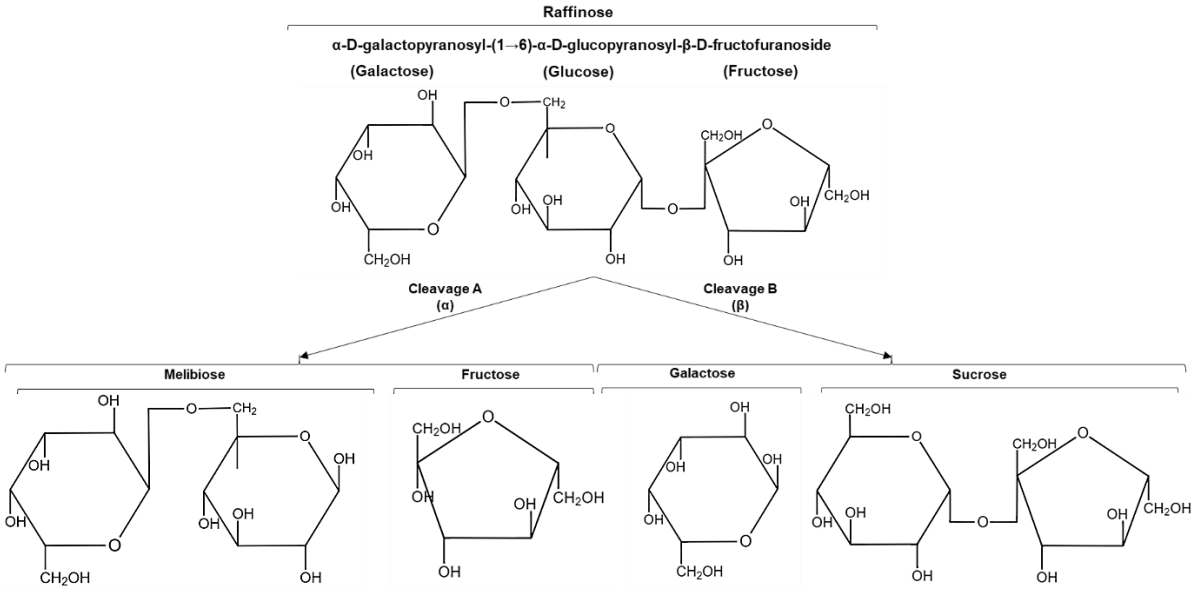

Figure 3-4- Raffinose structure and possible cleavages (A and B)
Cleavage A: Hydrolysis produce the monosaccharide D-fructose and the disaccharide melibiose.
Cleavage B: Hydrolysis produce the monosaccharide D-galactose and the disaccharide sucrose.

This trisaccharide is very common in plant seeds, leaves, stems, and roots. As is evident from its structure, it is a nonreducing sugar, as its anomeric carbon atoms are involved in glycosidic bonds (110, 111).

Raffinose was the chosen oligosaccharide standard, since being composed of three sugars linked by both an α and β bond, it can demonstrate the potential of solid acids to hydrolysate lignocellulosic biomass sugars. To determine the effect of IERs on the hydrolysis of raffinose, the treatments were carried out under 140ºC, a reaction time of 1h, and catalyzed by four resins that differ in their constitution. Their efficiency in monomers conversion was calculated and compared to the conventional catalyst H_2SO_4 4% $g.g^{-1}$ (concentration used in quantitative acid hydrolysis). The results are presented in Figure 3-5

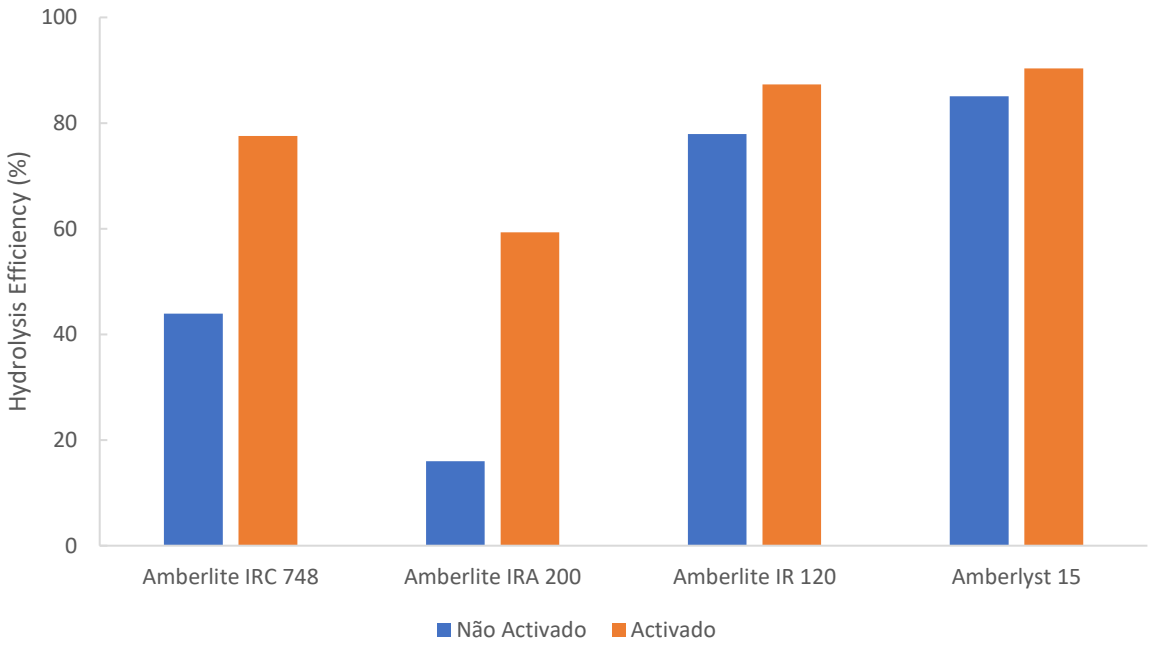

Figure 3-5-Raffinose hydrolysis efficiency for different IERs activated and non-activated

The main characteristic where was noted a great influence on the variation in hydrolysis efficiency was the ionic form present in each resin. While in Amberlite IR 120 and Amberlyst 15 not activated there is an ionic form H + and the efficiencies reached 77.9% and 85.0%, respectively, in Amberlite IRC748 and Amberlite IRA200 with an ionic form Na^+, efficiencies only reached 43.9% and 16.0%, respectively. This characteristic can be observed by the pH measurement presented in Figure 3-6, where it can be concluded that the ion exchange with resins carrying an H^+ form, the medium is acidified, leading to greater efficiencies, while resins in the Na^+ form tend to turn the medium alkaline, decreasing the efficiency of hydrolysis.

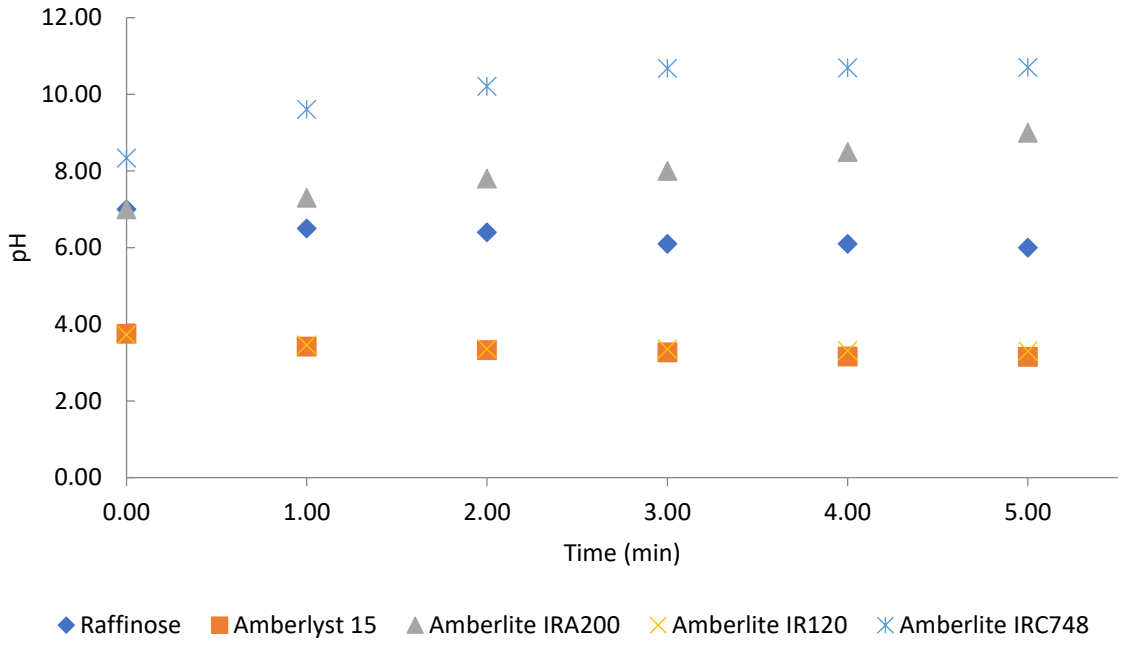

Figure 3-6-pH variation of raffinose catalyzed by different IERs for 5 minutes.

It should be noted that although Amberlite IR120 has, theoretically, a total Exchange capacity value higher than Amberlyst 15 (Table 2.1), both the pH decreased faster and hydrolysis was more efficient in the latter. This fact can be explained by the macroporosity existing in Amberlyst 15 which makes ion exchange faster compared to the gel structure present in Amberlite IR120 (112).

Regarding Amberlite IRC748 and IRA200 resins, it is possible to notice that despite the latter having a higher total Exchange capacity value (Table 2.1), the former presents a higher ion exchange speed with the medium, since the pH increased faster. This is possibly due to the iminodiacetic acid functional group present in Amberlite IRC748 which is also reflected in its high moisture-holding capacity.

To reverse the basicity of the resins with Na^+ ionic form and increase the acidity capacity of the resins that were already in the H^+ ionic form, it was decided to perform activation, in all the established catalysts, with H_2SO_4. Since this activation is carried out by placing the resins in H_2SO_4 4% $g.g^{-1}$ aqueous solution, both Amberlite IRC748 and IRA200 will perform an ion exchange with the medium, replacing the Na^+ ions with H^+ ions from the strong acid present. The Amberlyst 15 and Amberlite IR120 resins will also perform exchanges with the medium and thus increase the amount of H^+ linked to the functional groups. After this activation, it can be observed that Amberlite IRC748 and IRA200 experienced a huge improvement in their efficiency, increasing 33.6% and 43.3%, respectively, while Amberlite IR120 and Amberlyst 15 resins, despite increasing their efficiencies, only increased 9.4% and 5.3%, respectively. The conversion of the ionic form into H^+ is crucial because the acidity of the medium is a factor directly influenced by this characteristic, which in the sequence is essential in carrying out hydrolysis.

Analyzing these values and comparing them with the efficiencies obtained, the hydrolysis efficiency does not increase linearly concerning the number of BAS, as shown in Figure 3-7.

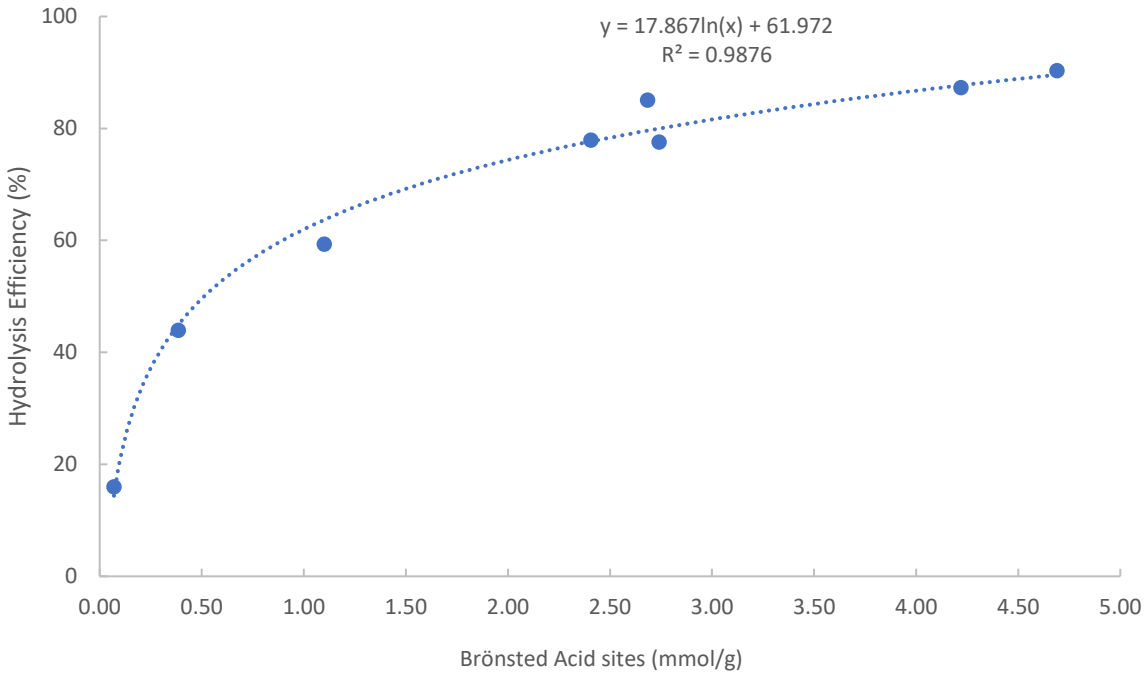

Figure 3-7-Correlation between raffinose hydrolysis efficiency and BAS.

In Figure 3-7, where the dependent variable is the BAS, a logarithmic behavior tendency is observed for the efficiency. The coefficient of determination is 0.9876, revealing a good fit. This means that efficiency tends to increase less and less as the number of H^+ protons present increases. Amberlite IRC748 has a greater ability to accept H^+ protons during activation when compared to Amberlite IRA200, however less hydrolysis efficiency can be observed when compared to Amberlyst 15 not activated, even with a similar number of BAS. Amberlyst 15 not activated with 2.68 mmol.g-1 has an efficiency of 85.1% while Amberlite IRC748 with a higher value of 2.74 $mmol.g^{-1}$ only achieves an efficiency of 77.6%. Analyzing this data, it can be concluded that, for the hydrolysis of raffinose, the functional group of sulfonic acid present in Amberlyst 15 can achieve better hydrolysis efficiencies concerning H_2SO_4 than the selective chelating iminodiacetic acid present in Amberlite IRC748 for an equivalent value of BAS.

3.2.3 Catalysts reutilization on raffinose hydrolysis

One of the major disadvantages when using mineral acids such as H_2SO_4 in lignocellulosic biomass hydrolysis is the fact that it is not possible to recover and reuse it. On the other hand, solid acids have the advantage of being easily recovered from a liquid medium by simple filtration. However, to really represent an advantage over the conventional method, resins need to have the ability to be reused without the need to reactivate/regenerate whenever used. For this reason, a study of the efficiency of the resins was carried out over 3 reutilizations, which can be seen in Figure 3-8.

Analyzing the plot, it can be observed practically no efficiency loss with Amberlyst 15, Amberlite IR120, and Amberlite IRC748 resins, throughout the three reutilizations Amberlyst 15 and Amberlite IR120 only lost 0.6% and 0.5%, respectively. which is within the admissible experimental error. In the case of Amberlite IRA200, an efficiency loss of 3.6% has been noticed, with a peculiar increase of 2.9% from the first to the second reuse. In conclusion, three of the four resins tested show potential for reutilization, at least three times, without losing their raffinose hydrolysis capacity. However, only Amberlyst 15 and Amberlite IR120 showed, not only a good potential for reuse but also high values of hydrolysis efficiency when compared to the conventional method with H_2SO_4.

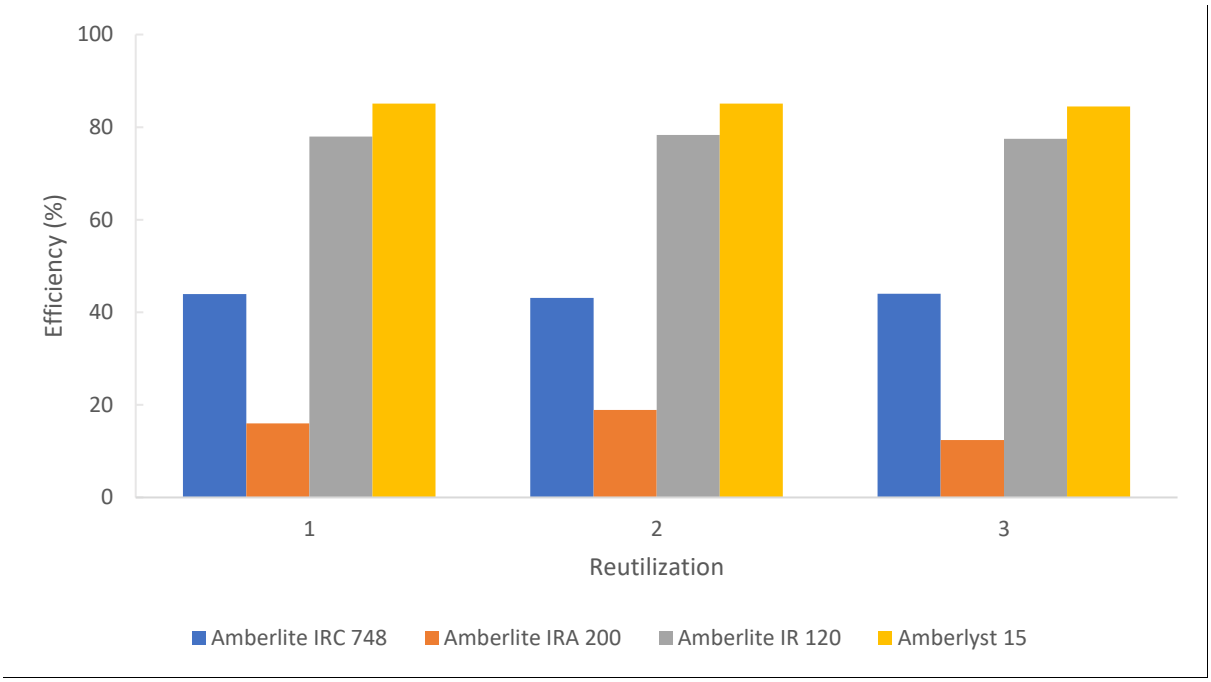

Figure 3-8-Efficiency of the IERs tested through reutilizations

3.3 Diluted acid hydrolysis

3.3.1 Diluted acid hydrolysis catalyzed by mineral acids

The chemical composition of hemicellulosic hydrolysates varies according to the raw material used, the type and concentration of catalyst, as well as the temperature and duration of the process. In this section, it is described the optimization of the dilute acid hydrolysis of ER and Miscanthus.

During the hydrolytic process, in addition to the sugars resulting from the polysaccharide fractionation, some compounds are also formed resulting from the degradation of hemicelluloses (acetyl groups), monosaccharides and partial degradation of lignin. Therefore, the various operating conditions that influence the process must be controlled and, if possible, optimized to maximize the concentrations of sugars and minimize the formation of degradation products that limit the use of hydrolysates as a culture medium (7, 41).

The acid concentration and the duration of the treatment are especially relevant and, usually, the most studied parameters (41). Since the effect of these variables is interdependent, their study must be carried out simultaneously.

In this work, for the optimization of the acid concentration and hydrolysis time, experimental statistical planning of Doehlert for two factors was used, in which the concentration of sulfuric acid was varied between 0 and 2% or 0 and 4% and the time between 0 and 240 min or 0 and 120min, for a temperature of 130 ºC. The choice of the sulfuric acid concentration range is justified by the fact that values above 4% make the method too expensive.

Table 3.1 and Table 3.2 present the matrix of the experiments carried out and the respective responses: concentrations of monosaccharides, acetic acid, and degradation products

The value of the modified combined severity factor (mCS) was calculated for each test, which is an empirical parameter that includes the effects of temperature, time, and acidity as explained before. This factor allows comparing tests performed under different operational conditions. The mCS ranged between 2.96 and 5.42 for the eucalyptus essays and between 2.66 and 5.42 for the Miscanthus ones. Since the variation in reaction time is greater in the eucalyptus and the variation in acid is greater than the Miscanthus, the value of mCS is similar between them.

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Table 3.1 Monosaccharides, acetic acid, and degradation products concentrations (g L^{-1}) obtained in the hydrolysis of ER extracted with dilute acid for different acid and time conditions, according to the Doehlert matrix.

Essays	Variables					Responses									
	Coded		Real												
	X_1	X_2	H_2SO_4 (%)	Tempo (min)	*mCS*	Glc	Xyl	Ara	TS	HAc	HLev	Furfural	HMF	TI	TS-TI
A1	0,000	0,000	1.00	120.00	4.97	5.28	16.64	5.46	27.38	3.97	0.42	0.59	0.34	5.31	22.07
A2	0,000	0,000	1.00	120.00	4.97	4.65	15.08	4.71	24.43	3.54	0.35	0.53	0.30	4.73	19.71
B1	1,000	0,000	2.00	120.00	5.27	6.21	16.09	5.98	28.28	3.71	0.80	1.06	0.24	5.81	22.46
B2	1,000	0,000	2.00	120.00	5.27	5.63	15.35	4.65	25.63	3.68	0.74	1.05	0.23	7.32	18.32
C1	-1,000	0,000	0.00	120.00	2.96	2.06	1.85	1.62	5.54	0.90	0.00	0.00	0.10	1.94	3.60
C2	-1,000	0,000	0.00	120.00	2.96	2.09	1.89	1.61	5.59	0.91	0.00	0.00	0.09	1.96	3.62
D1	0,500	0,866	1.50	223.92	5.42	5.86	16.21	6.03	28.09	3.83	0.79	1.25	0.25	6.12	21.97
D2	0,500	0,866	1.50	223.92	5.42	5.61	16.44	4.93	26.97	3.94	0.78	1.27	0.25	8.04	18.94
E1	-0,500	-0,866	0.50	16.08	3.80	2.87	4.24	5.22	12.33	1.38	0.00	0.00	0.00	1.38	10.94
E2	-0,500	-0,866	0.50	16.08	3.80	2.53	4.36	4.71	11.59	1.43	0.00	0.00	0.00	1.43	10.16
F1	0,500	-0,866	1.50	16.08	4.27	4.52	13.58	5.50	23.59	3.85	0.00	0.00	0.00	3.85	19.75
F2	0,500	-0,866	1.50	16.08	4.27	4.41	14.26	5.44	24.12	4.05	0.00	0.00	0.00	4.05	20.07
G1	-0,500	0,866	0.50	223.92	4.94	4.21	11.63	4.67	20.51	3.26	0.00	0.31	0.28	6.69	13.82
G2	-0,500	0,866	0.50	223.92	4.94	4.13	8.73	5.09	17.94	3.06	0.00	0.27	0.27	3.60	14.34

mCS – Modified combined severity; Glc – Glucose; Xyl – Xylose; Ara – Arabinose; TS – Total Sugars; HAc – Acetic acid; HLev – Levulinic Acid; HMF – Hydroxymethylfurfural; TS-TI - Difference between total sugars and total inhibitors; TI – Total inhibitors (Acetic acid, levulinic acid, furfural, and HMF).

Table 3.2- Monosaccharides, acetic acid, and degradation products concentrations (g L^{-1}) obtained in the hydrolysis of Miscanthus extracted with dilute acid for different acid and time conditions, according to the Doehlert matrix.

Essays	Variables					Responses								
	Coded		Real											
	X_1	X_2	H_2SO_4 (%)	Tempo (min)	*mCS*	Glc	Xyl	Ara	TS	HAc	Furfural	HMF	TI	TS-TI
A1	0,000	0,000	2.00	60.00	4.97	4.05	33.14	3.86	41.06	5.73	1.30	0.01	7.05	34.01
A2	0,000	0,000	2.00	60.00	4.97	4.21	33.47	4.23	41.90	5.93	1.00	0.00	6.94	34.97
B1	1,000	0,000	4.00	75.00	5.37	6.70	31.52	4.36	42.57	6.49	2.37	0.02	8.88	33.69
B2	1,000	0,000	4.00	75.00	5.37	6.52	31.01	4.12	41.66	6.33	2.41	0.02	8.76	32.90
C1	-1,000	0,000	0.00	60.00	2.66	0.80	0.45	0.00	1.25	0.75	0.00	0.00	0.75	0.50
C2	-1,000	0,000	0.00	60.00	2.66	0.65	0.00	0.00	0.65	0.50	0.00	0.00	0.50	0.15
D1	0,500	0,866	3.00	111.96	5.42	8.08	24.29	3.90	36.27	6.60	3.40	0.05	10.05	26.22
D2	0,500	0,866	3.00	111.96	5.42	7.98	25.39	4.10	37.48	6.82	3.92	0.04	10.78	26.70
E1	-0,500	-0,866	1.00	8.04	3.80	0.36	4.06	2.49	6.92	1.39	0.00	0.00	1.39	5.53
E2	-0,500	-0,866	1.00	8.04	3.80	0.35	5.53	2.53	8.42	1.73	0.00	0.00	1.73	6.69
F1	0,500	-0,866	3.00	8.04	4.27	0.99	20.41	3.82	25.22	4.42	0.00	0.00	4.42	20.80
F2	0,500	-0,866	3.00	8.04	4.27	0.96	19.80	3.56	24.32	3.72	0.00	0.00	3.72	20.61
G1	-0,500	0,866	1.00	111.96	4.94	4.18	30.68	3.95	38.81	5.54	1.10	0.03	6.67	32.14
G2	-0,500	0,866	1.00	111.96	4.94	4.17	32.92	4.15	41.25	5.71	1.23	0.01	6.96	34.29

mCS – Modified combined severity; Glc – Glucose; Xyl – Xylose; Ara – Arabinose; TS – Total Sugars; HAc – Acetic acid; HMF – Hydroxymethylfurfural; TS-TI - Difference between total sugars and total inhibitors; TI – Total inhibitors (Acetic acid, furfural, and HMF).

By analyzing the Table 3.1and Table 3.2, it can be observed that the highest concentration of total sugars is reached in the most severe condition (5.42) for eucalyptus and the second most severe condition (5.37) for Miscanthus. In the case of total inhibitors, the maximum concentration value was reached in the condition of the greatest severity (5.42 for both). Observing these data, we can say that sugars degrade faster in Miscanthus than in the ER.

Xylose is the main hemicellulose compound present in hydrolysates for most of the tests, being the monosaccharide that presents a greater increase in concentration. This increase in concentration results from the xylan hydrolysis of the raw material reaching, for the severity of 5.42, a concentration of about 16.33 $g.L^{-1}$, which corresponds to a yield based on xylan of the raw material of 75.8% for eucalyptus. For Miscanthus, for the severity of 4.97, is reached a concentration of about 33.31 gL^{-1}, which correspond to a yield of 91.51%. It should be noted that Miscanthus presents a higher concentration of xylan (19.2%) than eucalyptus (14.2%). However, given these efficiency and severity differences, obtaining a high yield in xylose, in eucalypt, seems to be hampered by its intrinsic nature, so it needs more stringent conditions than for Miscanthus.

Glucose is the second most important compound, but it appears in concentrations similar to arabinose. Contrary to what happens with xylose, these two monosaccharides do not undergo great variations in concentration with increasing severity. However, while glucose concentrations are due to low solubilization, arabinose concentrations are due precisely to the opposite. Arabinose has high solubilization, but the raw material presents low arabinan content. These facts, also verified in the hydrolysis with diluted acid from other lignocellulosic materials (108), demonstrate the selectivity of the treatment used for hemicelluloses, with low glucose concentration being a favorable aspect, since it favors the use of hydrolysate as a means of bioconversion of xylose into xylitol. It has been verified in other studies that high concentrations of this hexose can inhibit the metabolism of xylose in yeasts (113)

Furfural indicates the existence of xylose or arabinose degradation reactions since its dehydration leads to the formation of furfural. Similarly, the same happens with the degradation of hexoses in HMF and this in levulinic acid. As for these compounds, the concentration is always low, which may indicate that glucose is not significantly affected by varying conditions. However, in the eucalyptus essay, even if the mCS was similar, there was more concentration of HMF. This fact can lead to conclude that the reaction time (higher in this essay) may influence more this degradation than the acidity (higher in the Miscanthus essay).

In addition to the monosaccharides, the possibility of the presence of sugars also in the oligomeric form cannot be excluded, especially in the conditions of less severity. This may explain, in part, the low yields obtained, both in xylose, and the recovery of acetic acid, since if there are oligosaccharides, they may contain acetyl groups that are not being quantified in the analytical determinations carried out.

Bearing in mind that both produced hydrolysates are intended for the bioconversion of xylose to xylitol, it is necessary to select the conditions that allow maximizing the recovery of sugars, in particular xylose, and to minimize the formation of degradation compounds, since they are possible inhibitors of yeast metabolism. Therefore, the response to the difference between the total sugar concentration and the total inhibitors (TS-TI) was evaluated.

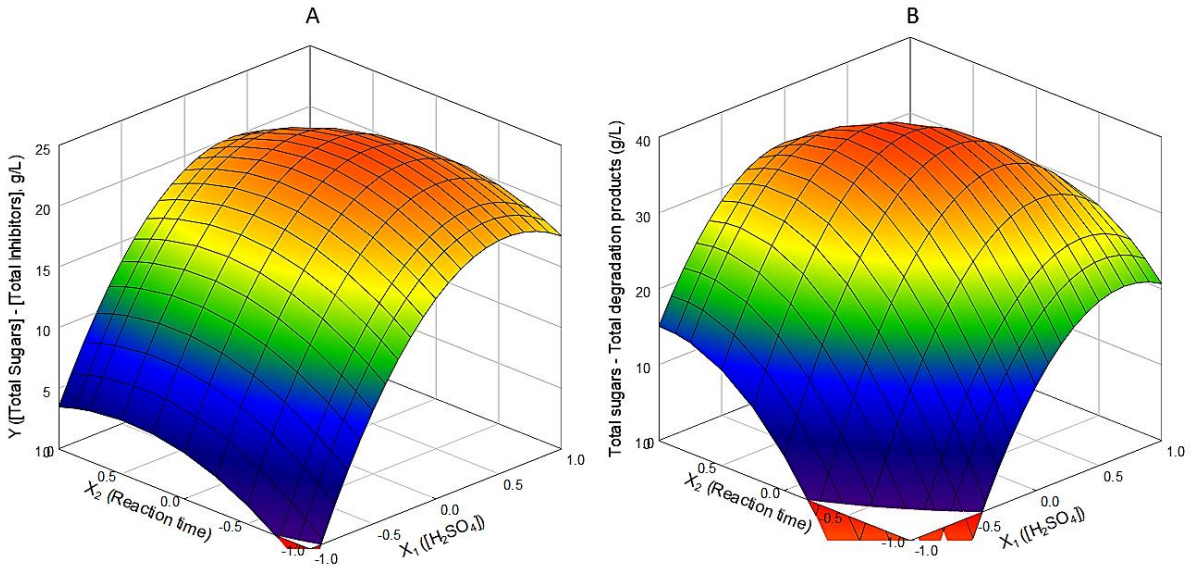

Figure 3-9- Response surfaces for the difference between the total sugar concentration and total inhibitors. A – ER; B-Miscanthus; X_1-H_2SO_4 concentration; X_2-Reaction time.

Based on the polynomial model, the optimal conditions, i.e. maximum monosaccharide recovery and minimal formation of acetyl groups and free degradation compounds in the hydrolyzate, were obtained by the linear program algorithm (MS solver Excel).

Among the various possibilities, the principle chosen was to maximize the direct difference between the concentration of sugars and inhibitors (TS-TI). The conditions under which this difference was maximized correspond to a reaction time of 127 min (X_2 = 0.061) and 1.46% (X_1 = 0.456) of acid for ER (Figure 3-9-A) and 77.11min (X_2 = 0283) and 2.50% (X_1 = 0.248) of acid for Miscanthus (Figure 3-9-B).These conditions correspond to a modified combined severity of 5.16 and 5.18, respectively. Table 3.3 shows the composition of both hydrolysates obtained under these conditions.

Table 3.3-Hydrolysates composition for the optimized conditions

mCS	5.16	5.18
Componentes	**Eucalyptus residues**	**Miscanthus**
-	($g \cdot L^{-1}$)	($g \cdot L^{-1}$)
Glucose	5.72	4.44
Xylose	18.06	35.87
Arabinose	5.90	4.30
Acetic acid	4.66	6.67
Levulinic acid	0.88	-
HMF	0,00	0.00
Furfural	0,00	1.34

The hydrolysates produced in the optimized conditions when compared to the hydrolysates produced in the 5.42 and 5.37 severity conditions, for which the highest sugar concentration was obtained for eucalyptus and Miscanthus, respectively, have higher xylose concentration, achieving a yield based on xylan of the raw material of 83.9% and 98.6%. Comparatively still to those hydrolysates, all inhibitors, except acetic acid, present lower concentrations in the hydrolysates obtained under the optimized conditions.

By analyzing the set of results presented, it can be concluded that the hydrolysis of ER with H_2SO_4 1.46% (w / w) at 130 ºC for 127 min (CS 5.16) and the hydrolysis of Miscanthus with H_2SO_4 2.50% (w / w) at 130 ºC for 77.11 min (CS 5.18) allowed:

- Obtaining hydrolysates rich in sugars with about 29.68 g. L^{-1} and 44.61 g. L^{-1} of monosaccharides, of which 18.06 g. L^{-1} and 35.87 g. $L^{-1}$are xylose, respectively.

- Obtaining hydrolysates with minimized concentrations of products potentially inhibiting microbial growth, such as furfural, HMF, acetic acid.

- The use of almost all the components present in the raw material, whose non-hydrolyzed fractions (essentially cellulose and lignin) can be used later for different applications, namely, to produce cellulosic ethanol.

3.3.2 Diluted acid hydrolysis catalyzed by solid acids

Since the IERs showed good results in the trisaccharide raffinose hydrolysis, dilute acid hydrolysis was performed using solid acids. However, acid hydrolysis directly in raw lignocellulosic biomass presents greater barriers in comparison to commercial sugars. Plants naturally evolved to withstand harsh external mechanical, thermal, chemical, and biological factors, and so are resistant to cell wall deconstruction These resistances are called biomass recalcitrance and it is well-known that cell wall recalcitrance varies among plant species and even within different phenotypes of the same plant (114). The DAH was performed on three types of lignocellulosic biomass, namely, ER, WS, and Miscanthus. The conditions employed were the same as those studied in raffinose hydrolysis. (140ºC and a reaction time of 60 minutes). Figure 3-10 shows the hydrolysis yield achieved.

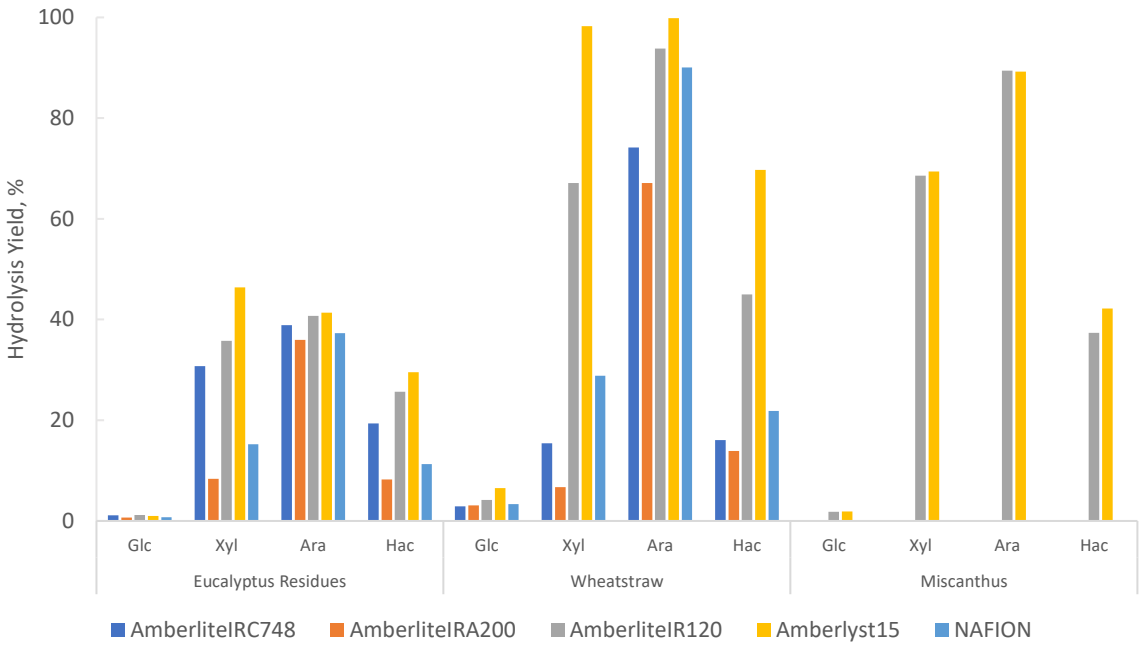

Figure 3-10- Hydrolysis yield based on the constitution of the raw material (Figure 3-2)

Analyzing Figure 3-10, it can be observed, as occurred in the optimization of dilute acid hydrolysis using H_2SO_4, the use of solid acids also demonstrates selectivity for hemicellulose, hardly affecting glucan. Furthermore, the hydrolysis yield of xylose concerning the initial xylan of the ER raw biomass is lower when compared to Miscanthus and even lower when compared to WS. This hydrolysis divergence can lead to assuming that the recalcitrance of the ER is superior to that of the other two biomasses. This recalcitrance can be explained by the fact that the ER is a hardwood and the other two biomasses are grasses, which is reflected in their initial composition (109).

Examining the composition of raw biomasses, ER contains a high percentage of lignin (22.1%) and a high ratio of arabinan (2.4%) and acetyl groups (5.8%) in relation to the initial xylan (14.2%). Lignin is found around hemicellulose, serving as a barrier, and arabinan and acetyl groups are branches directly linked to xylan (side chains) (115), which hinder access to it during treatments and explain the need for greater severity. Miscanthus, despite having a higher value of lignin (23.9%), has a lower ratio value between the side chains (1.8% of arabinan and 4.7% of acetyl groups) and the initial xylan (19.2%), which justifies not needing a severity as high as eucalyptus residue. Despite being a grass like WS, does not show values of yield as high as this one, since this biomass only has 18% of lignin and the lowest ratio between the side chains (3% of arabinan and 2.5% of acetyl groups) and the initial xylan (18.1%).

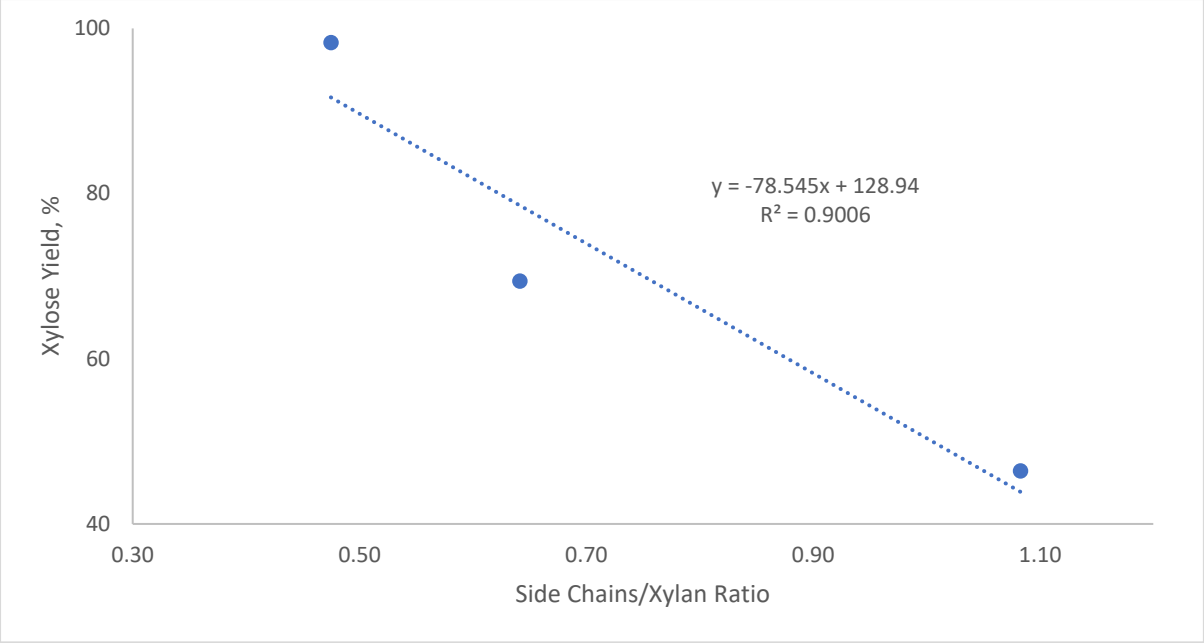

Figure 3-11- Correlation between Side Chains Ratio relatively to initial Xylan and Xylose Yield.

It should also be noted that the hydrolysis yield of acetyl groups in the ER varied between 8.2% and 29.6%, in the WS between 45.0% and 69.7% and in the Miscanthus between 37.3% and 42.2%, which demonstrates that a lower hydrolysis of this compound is cause for a more difficult access to xylan and thus decrease the xylose yield of the treatment.

At 140ºC and 60 minutes of reaction time conditions and based on the BAS of the activated IERs, it was calculated and determined, as shown in Table 3.4, the treatment mCS for each catalyst.

Table 3.4- Modified combined severity of the treatment at 140ºC during 60 minutes for the tested activated catalysts

Catalyst	mCS
Amberlite IRA200	4.30
Amberlite IRC748	4.70
Amberlite IR120	4.88
Amberlyst 15	4.93

Observing Table 3.4, the most severe mCS belongs to Amberlyst 15, which has the largest number of BAS and the lowest severity belongs to Amberlite IRA200, which has the least number of H^+ protons to donate. This fact is reflected in the hydrolysis yields of each lignocellulosic biomass. In the ER treatments, the lowest xylose yield was obtained, of only 8.37% for Amberlite IRA200 and the highest yield for Amberlyst 15, of 46.4%. The same was observed for the other biomasses, where higher mCS demonstrated better hydrolysis yields, reaching 98.3% in WS and 69.4% in Miscanthus. It should also be noted that the IER, Nafion NR50, which is also considered a superacid, was tested in these essays, for ER and WS. This resin, despite having a sulfonic acid functional group like three of the other tested resins, exhibit a different matrix composition of perfluorinated ether vinyl. Nevertheless, when compared with Amberlyst 15 or Amberlite IR120, it showed significantly lower xylose hydrolysis yields,

of 15.3% for ER and 28.9% for WS. Due to these results and the high cost of this resin, it was decided to discard its use in future trials.

Of the three biomasses studied, only WS is close to the optimal severity for complete hemicellulose hydrolysis since it reaches almost 100% of xylose and arabinose yield. ER and Miscanthus are still far from optimum yield and for this reason hydrolysis kinetics was carried out, where the reaction time was varied between 30, 60.90, 105, and 120 min for the two best-performing resins whose mCS are shown in Table 3.5.

Table 3.5-Modified combined severity values for the Amberlyst 15 and Amberlite IR120 treatments at 140ºC for different reaction times

Catalyst	Reaction time(min)	mCS
Amberlite IR120	30	4.58
	60	4.88
	90	5.06
	105	5.12
	120	5.18
Amberlyst 15	30	4.63
	60	4.93
	90	5.10
	105	5.17
	120	5.23

Figure 3-12 allows claiming that ER and Miscanthus, treated with a mCS of 5.18, only achieve 78.3% and 77.4% of xylan hydrolysis yield, respectively. The severity tested was not enough to reach xylose production optimal condition.

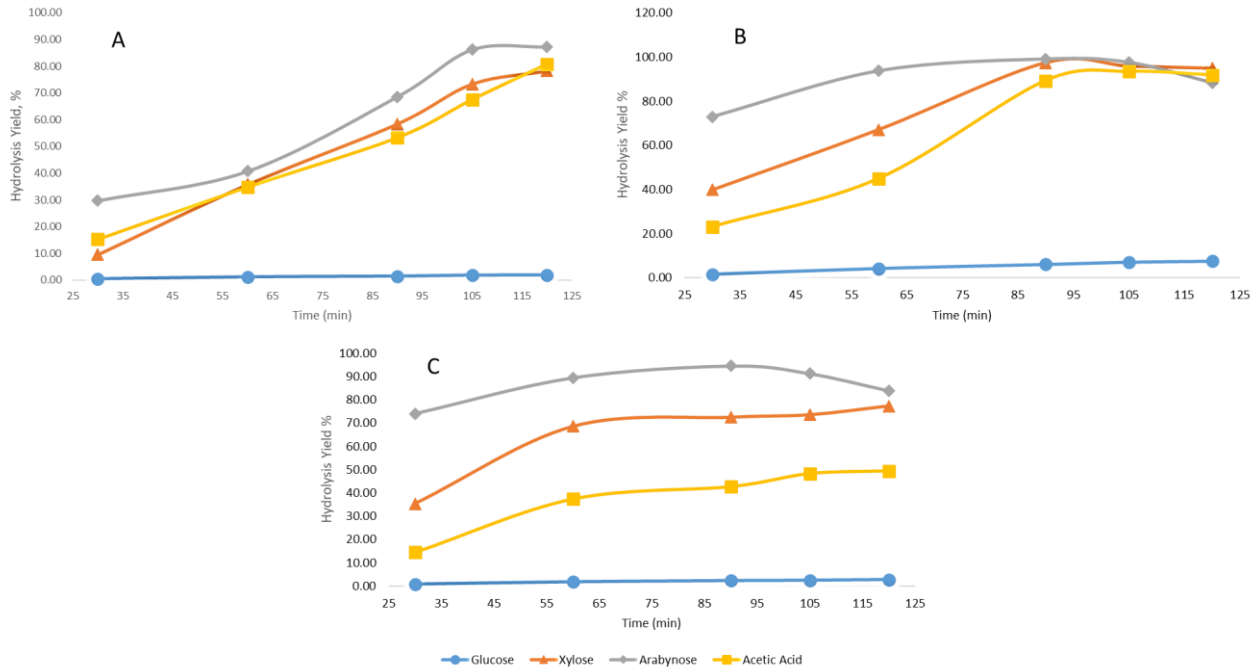

Figure 3-12-Hydrolysis yield based on the raw biomass of A-ER, B-WS, and C-Miscanthus catalyzed by Amberlite IR120.

Compared with the diluted acid hydrolysis catalyzed by H_2SO_4, where the optimum conditions were obtained for maximum sugar production and minimum inhibitors, with a mCS of 5.16 and 5.18, and a xylose hydrolysis yield achieved of 83.9% and 98.6% for ER and Miscanthus, respectively. It can be concluded that the Amberlite IR120 performance, under similar severity conditions, is inferior to the conventional process. WS, which is a lignocellulosic biomass with a lower recalcitrant effect than the previous ones, the optimal conditions are reached for a mCS between 5.06 and 5.12, since in the latter, a xylose concentration decrease has already been observed. This decrease is linked to the formation of pentoses degradation compounds like furfural. It should also be noted that ER, for a 5.18 mCS, an arabinan complete hydrolysis has not yet been reached, achieving 87.2%. Once again, the recalcitrant effect of this biomass is demonstrated. The total yield of this compound, for WS and Miscanthus, is between a mCS of 5.06 and 5.12, since in this interval there is already an arabinose decrease, showing some degradation to furfural. As arabinan, like acetyl groups, is a branch of xylan (115), it is natural to undergo hydrolysis and consequent degradation faster than xylan.

Amberlyst 15, which is an IER with a greater amount of BAS, achieve higher mCS values, under the same operating conditions. In addition to this greater amount of H^+ protons to donate, this resin has a macroporosity that makes ion exchange faster. These two allied characteristics reflect in the best hydrolysis yields shown in Figure 3-13.

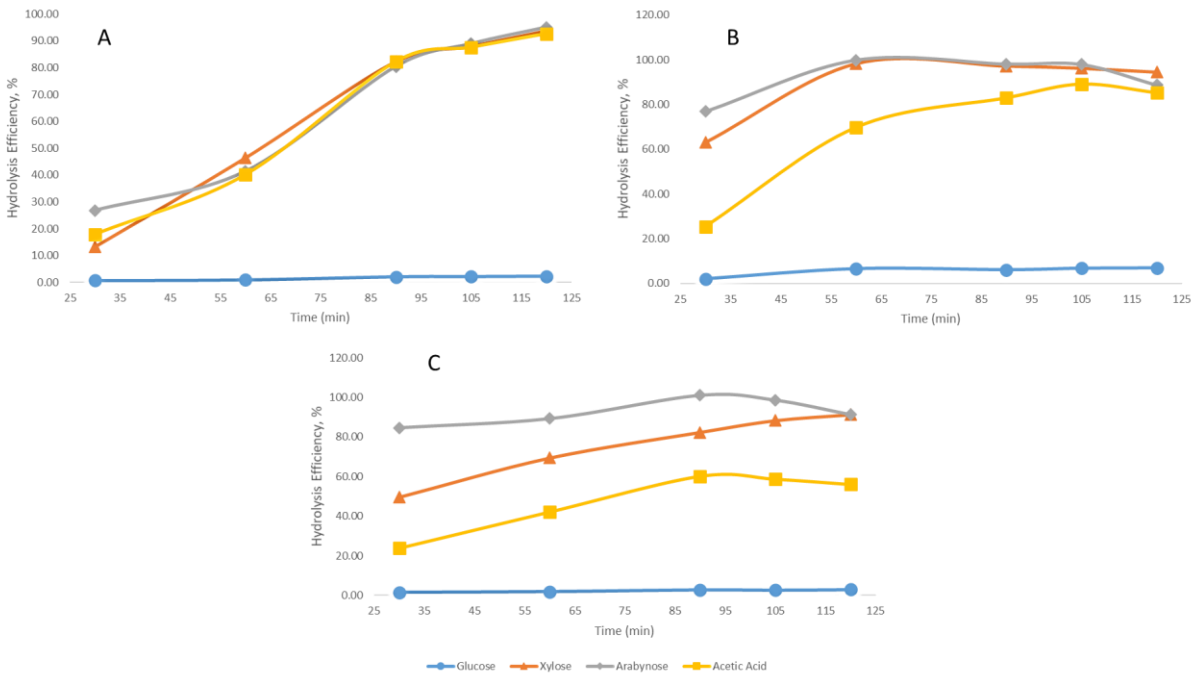

Figure 3-13- Hydrolysis yield based on the xylan of raw biomass of A-ER, B-WS and C-Miscanthus catalyzed by Amberlyst 15.

For ER and Miscanthus hydrolysis catalyzed by Amberlist 15, under a reaction time of 120 min, corresponding to a mCS of 5.23, there is an increase in xylose yield of 15.4% and 14.0%, respectively, concerning Amberlite IR120. For WS, the optimal value for both xylose and arabinose yield seems to be close to a mCS of 4.93, under 60 min reaction time, where yields of 98.2% and 99.9%, respectively,

are achieved. The arabinan hydrolysis for ER, catalyzed by Amberlyst 15, under the same reaction time as Amberlite IR120, also shows a higher yield, reaching a value of 95.1%. For Miscanthus, with a mCS of 5.10, equivalent to a reaction time of 90 minutes, a decrease of this compound is already noticed, due to degradation.

In conclusion, IERs, particularly Amberlyst 15, have the potential for dilute acid hydrolysis competing with H_2SO_4 hydrolysis efficiency. However, since this mineral acid has a more competitive cost than IERs, there is a need to recover the solid acids and reuse them for this method to be really competitive. When carrying out hydrolysis where the catalysts are mixed with raw biomass and water, there is a great difficulty with the separation between both solids after treatment, It is not possible to perform a simple filtration or decantation as done before when working with liquids.

Bearing this in mind, it was thought about the production of magnetized activated carbon, since activated carbon also shows good results in sugar yield, as can be seen in Table 1.3. Despite the magnetization of the coal has resulted, this process involves washing the catalysts in NaOH aqueous medium with a pH of 10-11, turning the catalyst alkaline and inefficient for sugar hydrolysis, reaching only 4.0% of xylose yield and 23.4% of arabinose yield for WS, as can see seen in Figure 3-14.

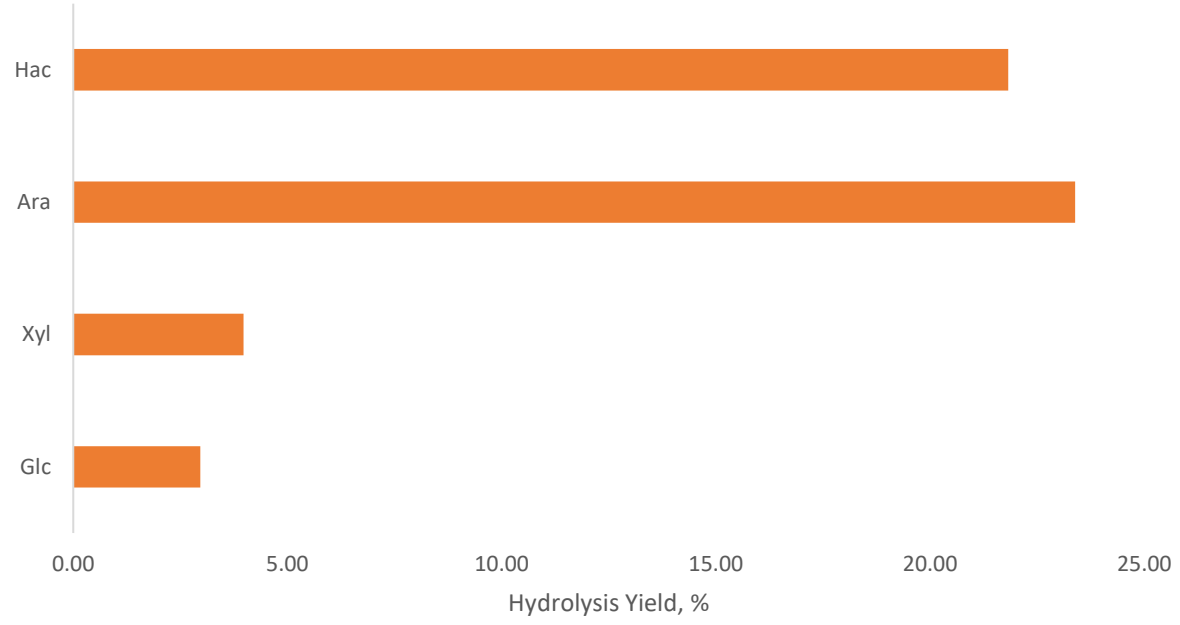

Figure 3-14- Wheat straw hydrolysis yield catalyzed by saccharose magnetic carbon

3.4 Batch oligosaccharides hydrolysis

3.4.1 Oligosaccharide production

Due to the difficulty of recovering the solid acids when performing DAH allied with the acquisition of better quality lignin and cellulose when performing a different pre-treatment before the application of the IER's, a two-step system was assembled, where the process begins with a pre-

treatment of autohydrolysis or organosolv to obtain the oligosaccharides liquor and then the IERs are applied in a post-hydrolysis to obtain monosaccharides. The two pre-treatments used have different functionalities. Autohydrolysis separates cellulose and lignin (solid phase) from hemicellulose (liquid phase), focusing on the yield of the latter, and organosolv separates cellulose (solid phase) from hemicellulose and lignin (liquid phase), focusing on lignin removal efficiency. Despite these differences, and a focus on hemicellulose being more appropriate for this work of recovering sugars from hemicellulose, we found it interesting to study the behavior of IERs in a medium where, in addition to oligosaccharides, solubilized lignin and ethanol will also be present. The lignocellulosic biomasses tested in this process were ER and WS.

3.4.1.1 Temperature profiles

ER and WS were subjected to autohydrolysis treatments under non-isothermal conditions (reaction was stopped when the reaction medium reached the desired temperature), for the final temperature of 190ºC. Autohydrolysis of ER under 190 ºC is optimized to yield mainly soluble oligosaccharides(116), and for that reason, this condition was applied for both ER and WS. The temperature and pressure profiles were registered for each treatment since they can be useful to determine the reproducibility of the treatments as mentioned before (Figure 3-15 and Figure 3-16).

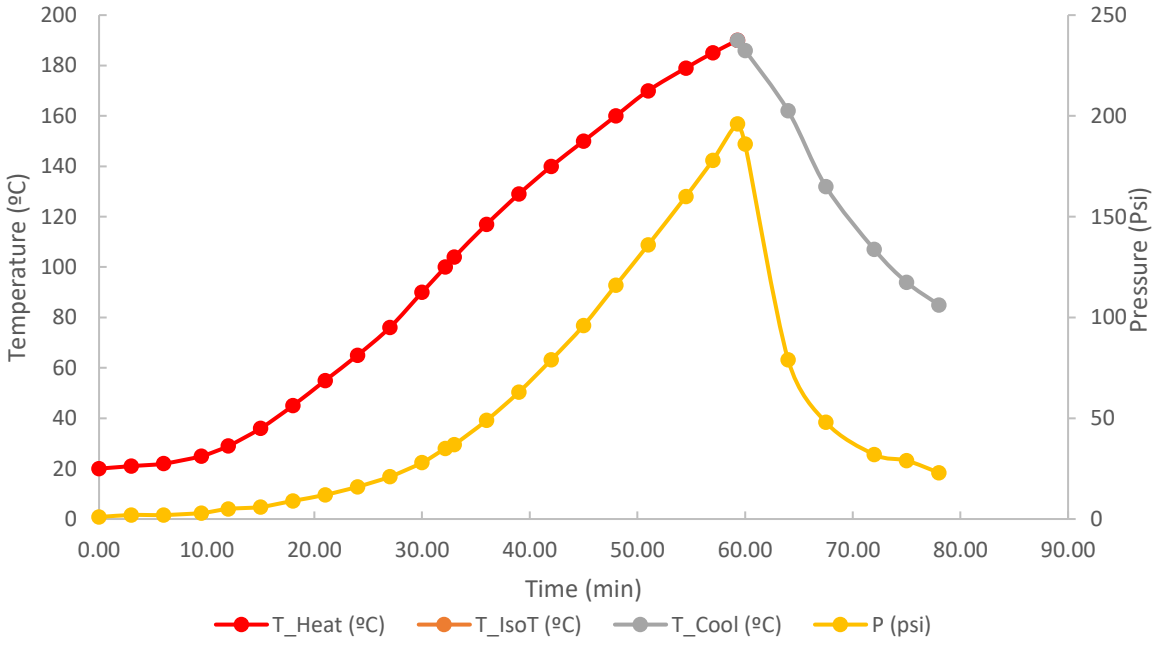

Figure 3-15- Temperature and pressure profile for autohydrolysis treatment of WS.

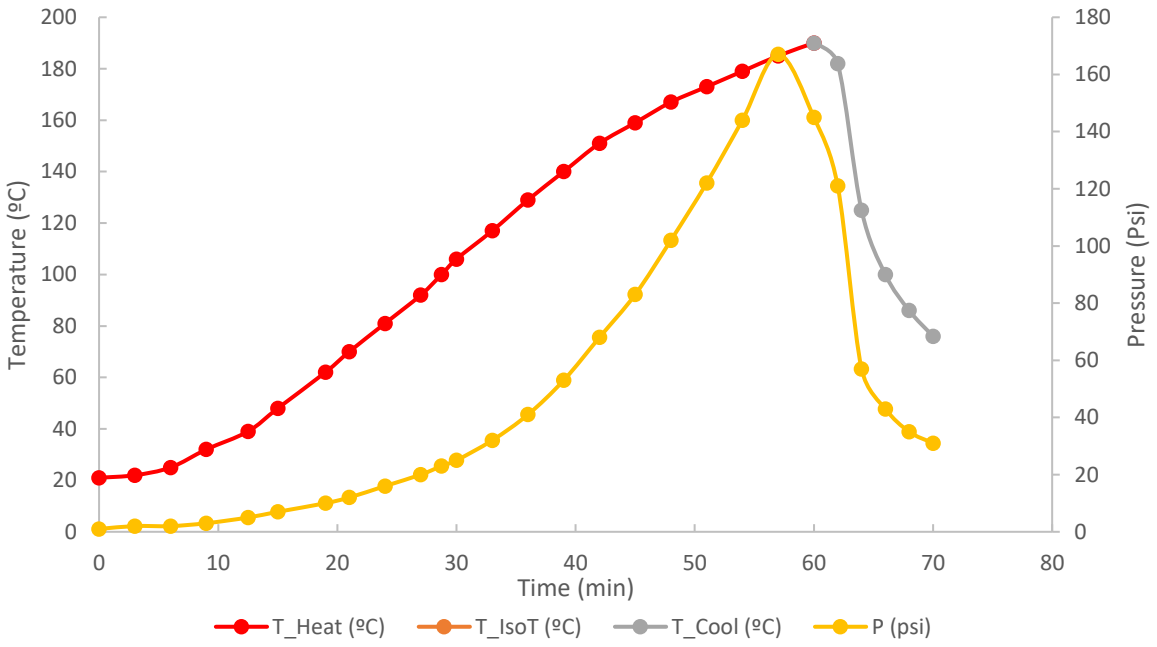

Figure 3-16- Temperature and pressure profile for autohydrolysis treatment of ER.

The temperature and pressure profiles represented in Figure 3-15 and Figure 3-16 were quite similar between them. For the temperature, the profiles showed an initial lag phase in the first 8-10 minutes, followed by a linear increase until reaching the final temperature. The pressure profiles are quite consistent among themselves with an exponential increase until the end of the assay.

ER and WS were subjected to a previously optimized organosolv treatment, for delignification, with 50% (w/w) ethanol at isothermal conditions for 2 hours, at a temperature of 190ºC (Figure 3-17 and Figure 3-18). For the WS treatment, temperature and pressure profiles were collected while for ER, unfortunately, due to some technical malfunctions the pressure could not be measured. These profiles can be useful to check the reproducibility of the treatments and to ensure that the different effects detected after treatment are due to the operational conditions used and not to abnormal variation of temperature and pressure during treatment, so the results obtained can be compared.

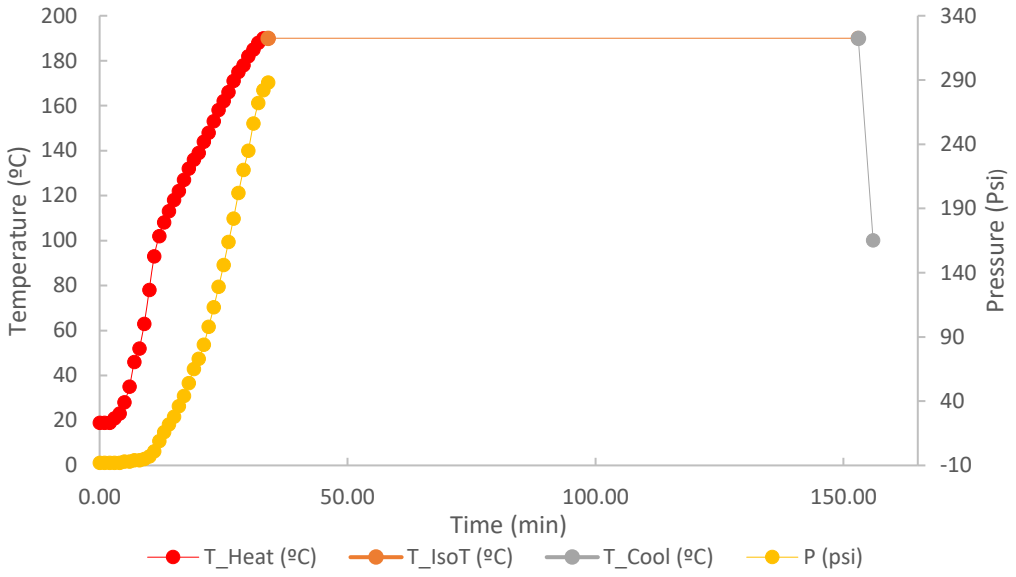

Figure 3-17- Temperature and pressure profile for organosolv treatment of WS.

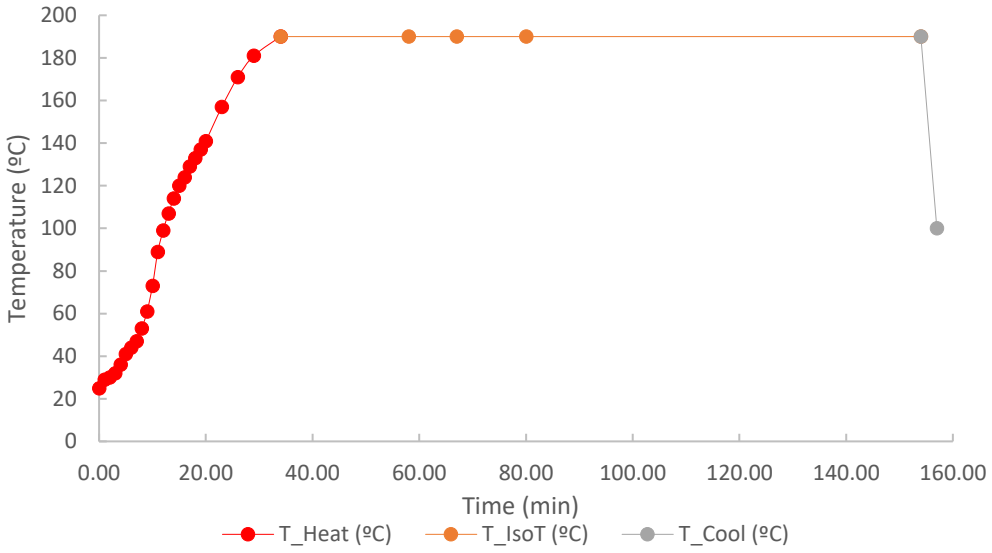

Figure 3-18- Temperature profile for organosolv treatment of ER

Figure 3-17- Temperature and pressure profile for organosolv treatment of WS.and Figure 3-18- Temperature profile for organosolv treatment of ER shows temperature and pressure profiles for organosolv treatment of WS and ER, respectively, at the temperature mentioned before. Temperature profiles are quite similar to each other. After an initial lag phase, there is a linear increase in the temperatures. There is a difference in the initial temperatures related to the fact that room temperature may differ day by day. Pressure profiles display an exponential increase, reaching almost 300 psi.

Comparing to the pressure and temperature profiles obtained for autohydrolysis, organosolv profiles present more deviations, which can probably be related to the behavior of a system where only water was added to the biomass *vs* a system where ethanol: water mixtures were used.

3.4.1.2 Composition of the liquid phases

Both treatments mentioned before, produce a liquid and a solid phase, however since the main goal of autohydrolysis and organosolv for this work is the production of hydrolysates with oligosaccharides, only this liquid phase will be discussed. Figure 3-19 shows the composition of these liquids.

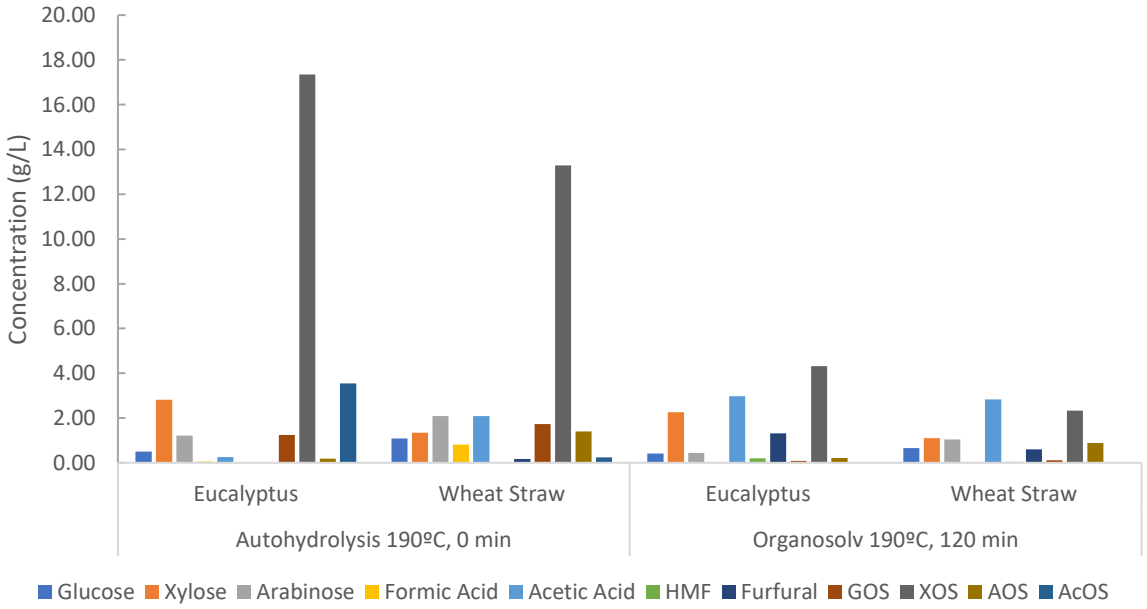

Figure 3-19- Composition (g/L) of the liquors obtained from autohydrolysis 190ºC and organosolv 190ºC, 120min of ER, and WS. GlcOS – gluco-oligosaccharides; XOS – xylose oligosaccharides; AOS – arabinose oligosaccharides AcOS – acetyl groups linked to xylooligosaccharides.

Regarding sugar monomers, in general, the concentration is low for both treatments. Glucose and arabinose concentrations are higher in WS reaching 1.08 g/L and 2.08 g/L, respectively, with autohydrolysis, and 0.66 g/L and 1.05 g/L, with organosolv. On the other hand, xylose presented higher values of concentration for ER, reaching 2.81 g/L with autohydrolysis and 2.45 g/L with organosolv. From the oligosaccharides present in the liquors, XOS were the saccharides present in higher concentration and resulted from the solubilization of xylan, the major component of the hemicelluloses present in the two lignocellulosic materials tested. Comparing ER and WS, about autohydrolysis performance, it is possible to tell that 190ºC is the temperature optimized to recover oligosaccharides, specifically for ER, since this biomass presented more 4.06 g/L of XOS concentration relatively to WS, even when the former is constituted with a higher percentage of xylan. Some authors claim to achieve 20 g/L and 12 g/L of XOS concentration with an optimal temperature of 210-215ºC treating WS and ER, respectively (117). Under these conditions, a higher XOS concentration value for WS was achieved, but a lower value for ER. Under 190ºC was achieved 17.35 g/L of XOS which is a way better yield than the one reported at 210-215ºC. However, both conditions cannot be completely comparable, since the reactor type used in both essays is not the same, and the heating profile is crucial to autohydrolysis process optimization. Analyzing the oligosaccharides concentration is possible to understand the different objective between the two pre-treatments since autohydrolysis can achieve way higher values

of XOS when compared to organosolv, where there is a significant amount of furfural produced, reaching 1.31 g/L for ER and 0.61 g/L for WS, which implies a lower recovery of pentoses (41). As it is explained in other articles, autohydrolysis leads to a liquid phase rich in hemicellulose-derived sugars or oligomers without causing significant dissolution of cellulose and lignin and when operating under optimized autohydrolysis conditions, most hemicelluloses can be recovered in liquors as XOS and xylose. (38) The approach with organosolv leads to lignin fragments suitable for a variety of purposes, and to delignified solids with improved susceptibility towards enzymatic hydrolysis but when this method is applied to raw lignocellulosic biomass, hemicelluloses can be just partially dissolved or converted into sugar-dehydration products such as furfural (118, 119).

This higher degradation can be explained by the difference in severity needed to provoke the delignification of biomass against the severity needed to solubilize the maximum amount of oligosaccharides, presented in Figure 3-20, where we can observe a difference of 1.15 in the severity factor between the two processes performed.

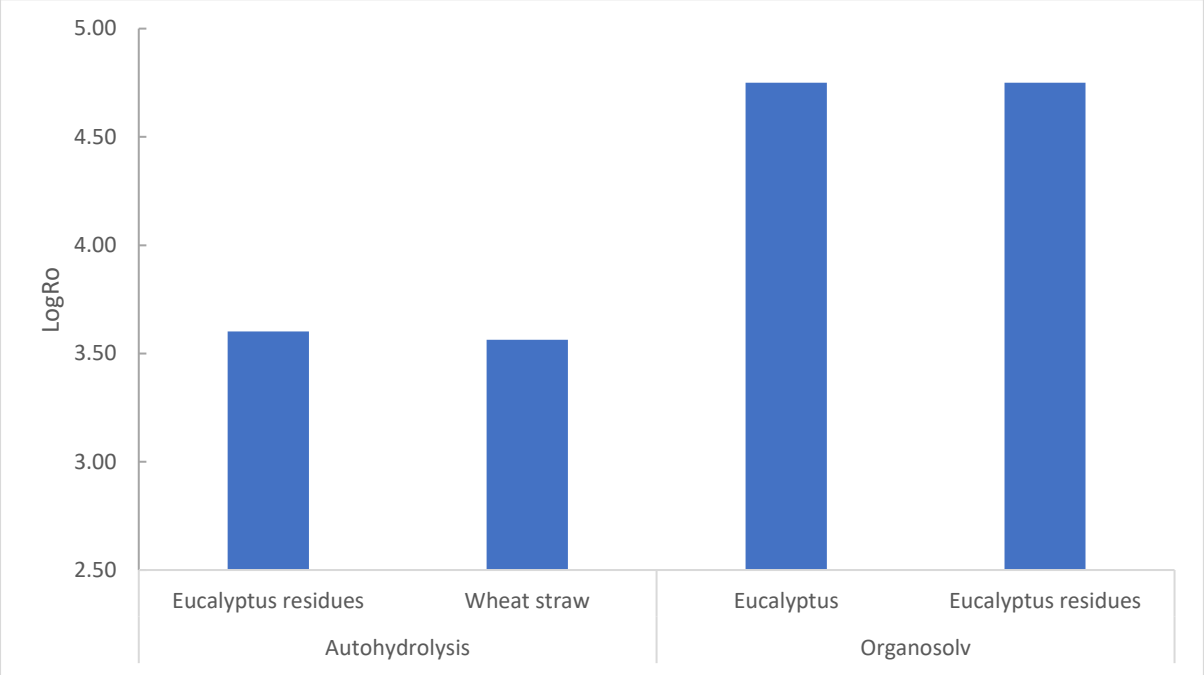

Figure 3-20- Severity factor (logRo) used in the treatments of ER and WS with autohydrolysis and organosolv processes.

3.4.2 Hydrolysis of autohydrolysis oligosaccharides

Oligosaccharides quantification was performed by applying conventional quantitative acid hydrolysis using H_2SO_4 4% (g / g) under a temperature of 121ºC and 60 min of reaction time, corresponding to a mCS of 5.12. Since, in the oligosaccharides hydrolysis catalyzed with IERs, the same conditions studied in both the hydrolysis of raffinose and in DAH, were adopted (under 140ºC and 60 min of reaction time), it was decided to test the same concentration of H_2SO_4 4% (g / g) under these conditions, which corresponds to a mCS of 5.57. The concentrations of monosaccharides obtained in the ER and WS liquors hydrolysis are shown in Figure 3-21.

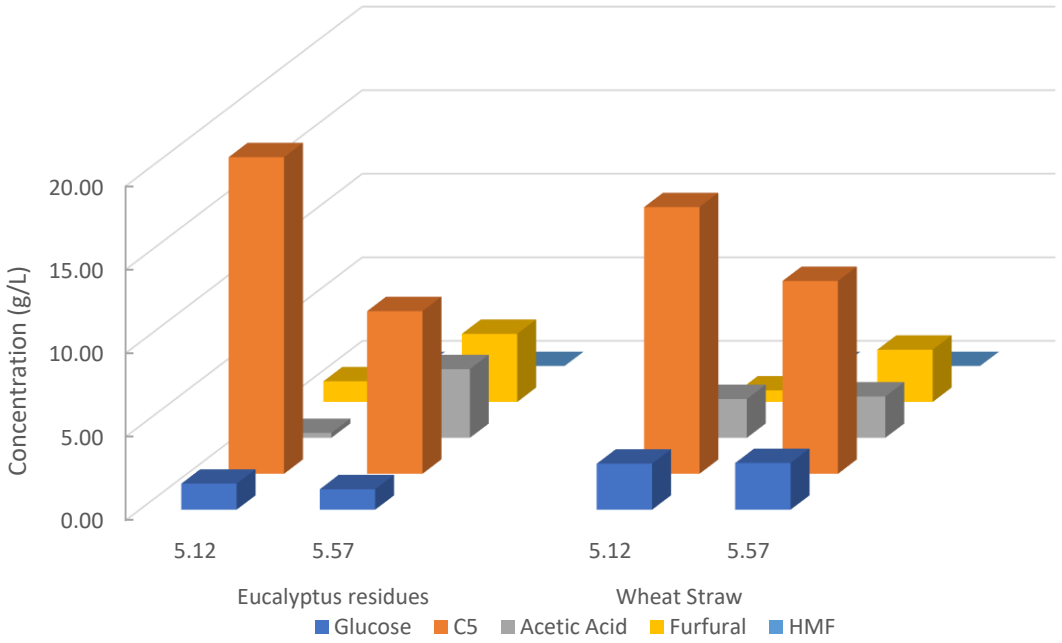

Figure 3-21- Monosaccharides concentration (g/L) obtained from the hydrolysis of ER and WS liquors, obtained performing autohydrolysis, catalyzed by H_2SO_4 with a mCS of 5.12 and 5.57. C5 stands for pentoses concentration (xylose + arabinose)

A high increase in the severity applied, also influence a high variation of monosaccharides concentration values for both tested feedstocks. In terms of glucose, there is not a very sharp variation from one condition to another, nor is there any degradation of this compound, as the concentration of HMF remains null. However, when analyzing the pentoses present in the ER liquor, from the severity of 5.12 to 5.57, 9.21 g / L is lost, which translates into the 2.84 g / L of furfural, and in WS liquor 4.42 g / L is lost, which translates into 2.43 g / L of produced furfural. Compared with DAH, the treatment of pre-treated oligosaccharides does not present as high a recalcitrance as raw biomass, since the lignin, which serves as a barrier, has already been broken and separated, offering a greater susceptibility of the oligosaccharides to acid hydrolysis. As can be seen from the data presented, this sudden variation in mCS caused a great increase in pentoses degradation to furfural. For this reason, conventional quantitative acid hydrolysis, with a mCS of 5.12, was used as a comparison for the tested IERs, which under conditions of 140ºC, 60 min correspond to the mCS present in Table 3.6.

Table 3.6- Calculated mCS values for the hydrolysis of oligosaccharides using activated and non-activated catalysts, at 140ºC, 60min (isothermal condition).

Catalyst	Activated	
-	**No**	**Yes**
Amberlite IR120	5.04	5.28
Amberlite IRA200	3.50	4.70
Amberlite IRC748	4.24	5.09
Amberlyst 15	5.08	5.33

As was tested in the standard oligosaccharide, raffinose, the ER and WS autohydrolysis liquors hydrolysis, was also carried out applying activated and non-activated solid catalysts. The efficiencies concerning the quantitative acid hydrolysis obtained are shown in Figure 3-21 and Figure 3-22.

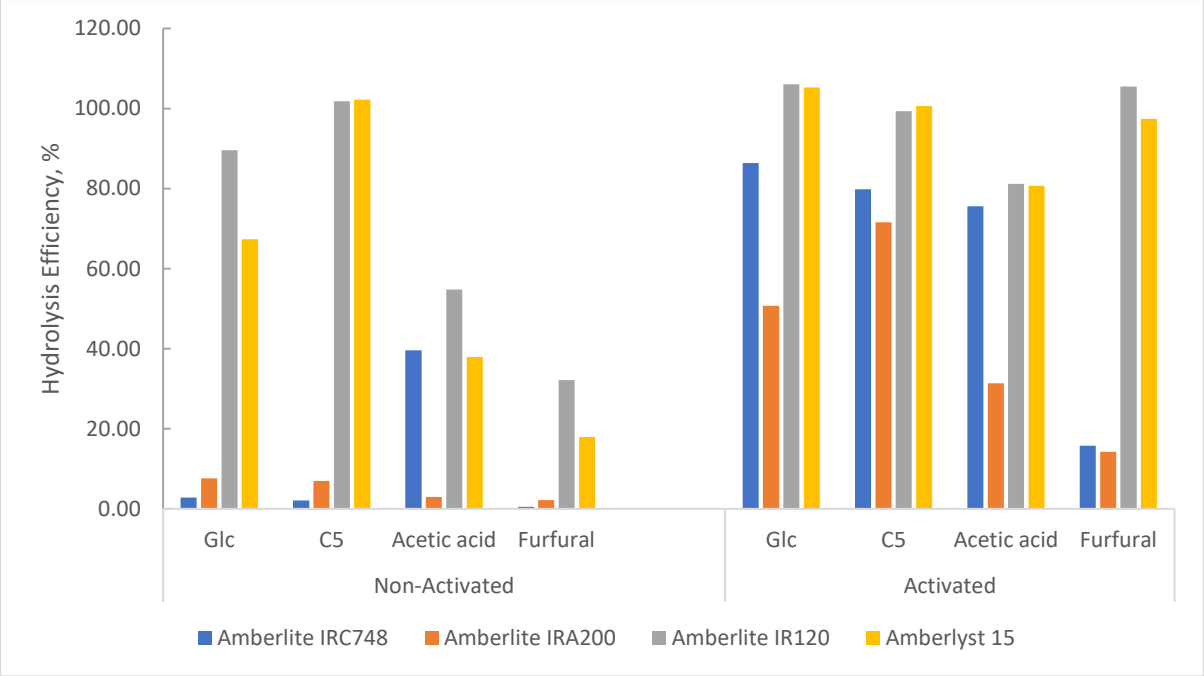

Figure 3-22- Autohydrolysis ER liquor hydrolysis efficiency with activated and non-activated solid catalysts.

Analyzing Figure 3-22, a large difference in efficiencies can be noticed between Amberlite IRC748 and Amberlite IRA200 concerning Amberlite IR120 and Amberlyst 15. After activation, the first two resins, achieve a great increase in efficiency, with an increase in C5 hydrolysis efficiency of 77.7% for Amberlite IRC748 and 64.6% for Amberlite IRA200. However, when compared with Amberlyst 15 and Amberlite IR120 these values are always lower, showing that the reaction mechanism of IERs is congruent with the discussion performed earlier when hydrolyzing raffinose. The resin's behavior is similar for both hydrolyzes of commercial oligosaccharides and hydrolysis of oligosaccharides from autohydrolysis liquor. Comparing the two best performing resins, it should be noted that both have good hydrolysis results, activated and non-activated. Amberlite IR120 and Amberlyst 15 not activated, with a mCS of 5.04 and 5.08, achieved C5 efficiencies higher than quantitative acid hydrolysis, reaching an additional 1.8% and 2.2%, respectively. The severity of the process, with the use of these catalysts, is slightly lower than the mCS of 5.12 applied in the conventional method, performing better since lower degradation values were obtained. Amberlite IR120 obtained 32.2% of the furfural produced when using H_2SO_4 and Amberlyst 15 only got 17.9%. When these resins are activated, the process mCS is 5.28 when catalysed with Amberlite IR120 and 5.33 when Amberlyst 15 is applied. This increase in severity is reflected in overall sugars hydrolysis. Amberlite IR120 and Amberlyst 15 show a slight increase in glucose efficiency of 6.0% and 5.3%, respectively, of the small amounts of glucan present in the liquor, compared to the conventional method. Still concerning this method, in the C5 hydrolysis, despite a higher mCS, Amberlyst 15 and Amberlite IR120 demonstrate similar behavior, obtaining efficiencies of

100.6% and 99.4%. This fact proves that the IERs, despite being in conditions of higher severity, the slower and more controlled release of H^+ protons into the medium, allows efficiency values similar to the quantitative acid hydrolysis catalyzed by H_2SO_4.

Figure 3-23 indicates that IERs have similar behavior for both ER and WS liquor hydrolysis. However, it should be noted that under conditions with a lower mCS, like when catalysed with Amberlite IR120 and Amberlyst 15 not activated, a higher furfural production than the one noticed in the ER, is obtained. This furfural increment can be justified by the fact that WS is a feedstock with a higher arabinan content than ER, and since this compound is a branch of xylan, it will undergo hydrolysis and subsequent degradation to furfural faster than xylan.

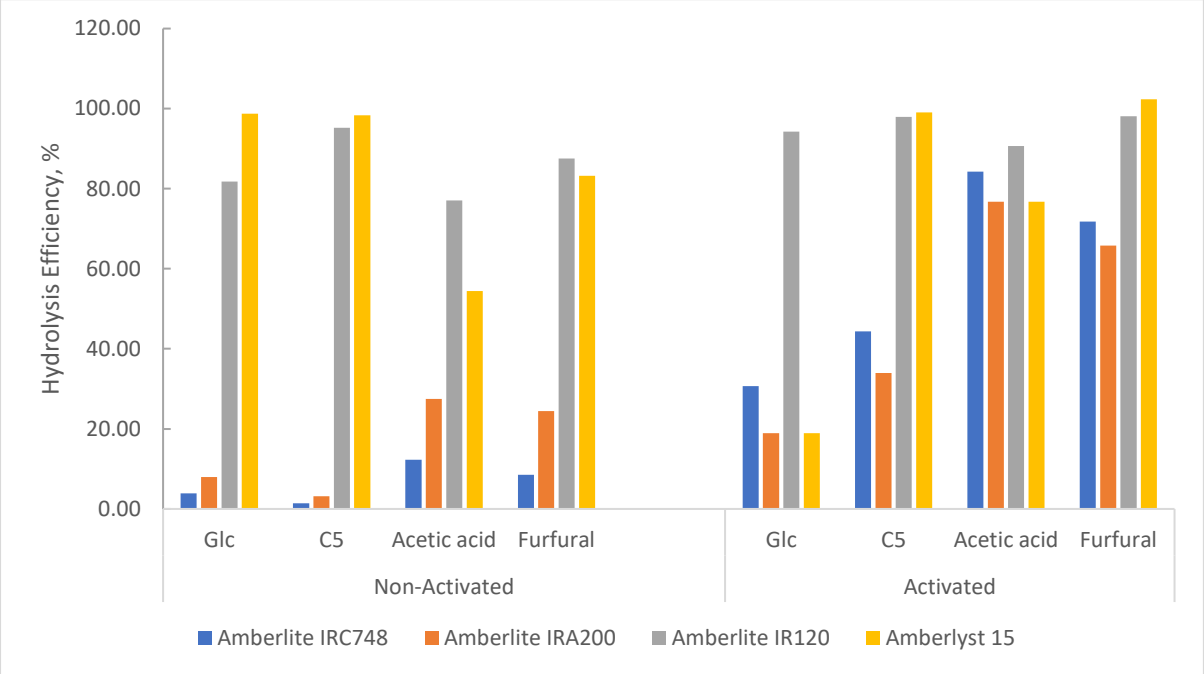

Figure 3-23- Autohydrolysis WS liquor hydrolysis efficiency with activated and non-activated solid catalysts.

In conclusion, as had been analyzed in the study of the commercial oligosaccharide raffinose, also for the autohydrolysis produced oligosaccharides it is possible to achieve great sugars hydrolysis when catalysed by IERs, namely Amberlite IR120 and Amberlyst 15. However, as discussed earlier, there is a need for these resins to demonstrate the ability to be reused without constant regeneration/reactivation. For this reason, was carried out a study on the reutilization of the two resins with better efficiency results.

Figure 3-24 demonstrates a completely different behavior of both IERs tested, during the 5 reutilizations, when catalyzing ER liquor and WS liquor. When ER liquor is used as feedstock, between the first and the third run, practically no performance is lost, occurring a slight decrease to 95.6% for Amberlite IR120 and 96.8% for Amberlyst 15. The greatest loss of performance is noted between the third and the fourth run, where a decrease of 15.7% and 14.4%, for Amberlite IR120 and Amberlyst 15, respectively, is observed. However, at the end of the fifth run, the resins still retain 77.92% and 81.26% of the initial performance. Though, when WS liquor is used as feedstock, the behavior is different.

During two reutilizations an acceptable performance is retained decreasing only 5.6% and 8.4% for Amberlite IR120 and Amberlyst 15, respectively. From the third run on, performance decline way further, dropping 49.0% for AmberliteIR120 and 52.9% for Amberlyst 15. At the end of the fourth run, only 26.5% and 24.8% of the initial performance was retained.

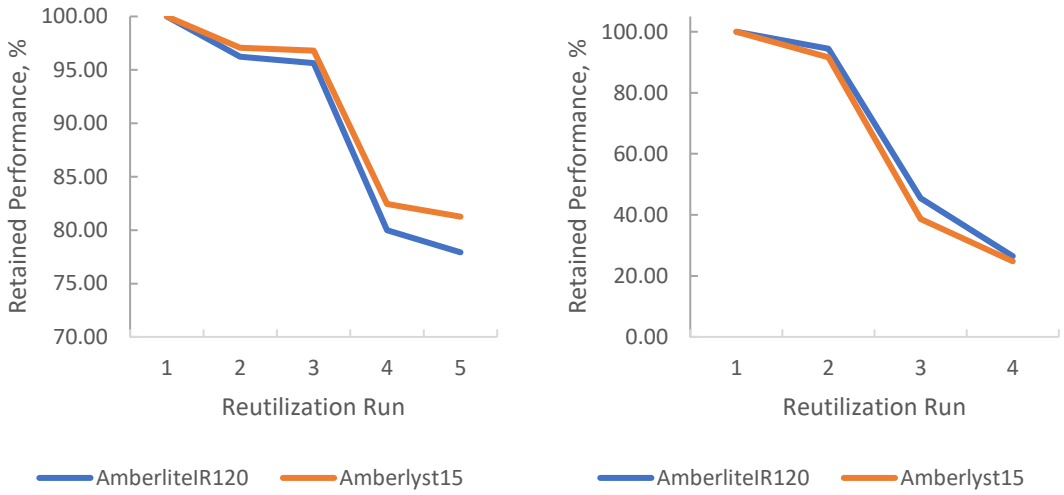

Figure 3-24-Retained performance of Amberlite IR120 and Amberlyst 15 C5 hydrolysis after 5 reutilizations on ER (left) and WS (right) liquor from autohydrolysis.

Compared with the repetitions performed with raffinose as feedstock, where performance was practically not affected during the reutilization, the experiments on autohydrolysis liquor, mainly in WS, obtained different results. Unlike commercial raffinose, lignocellulosic biomass feedstocks have a percentage of ash that refers to the inorganic matter present in raw biomass. ER, as can be consulted in Figure 3-2, only has 2.4% of ash content while WS has 9.7%. As is known, the apparent selectivity of any IER for a given metal depends on concentration (120), and existing higher concentrations of inorganic matter such as SiO_2, K_2O, Al_2O_3, Fe_2O_3, CaO, MgO and TiO_2 (compounds present in the WS ash) (121), the probability that the resin will lose the proton H^+ and be replaced by another is greater than in a medium where these compounds do not exist. The loss of performance over the runs is attributed to the ash concentration in the feedstock, which will interfere with the ion exchange of resins. It should also be noted that Amberlyst 15, due to its morphological characteristics achieves a faster ion exchange, which is reflected in a faster loss of performance in a higher concentration of ash, as can be seen in Figure 3-24.

3.4.3 Hydrolysis of organosolv oligosaccharides

The organosolv liquor, since this treatment is not the most suitable for obtaining oligosaccharides, simply the performance of the resins during reutilization was discussed. The retained performance along the runs can be seen in Figure 3-25.

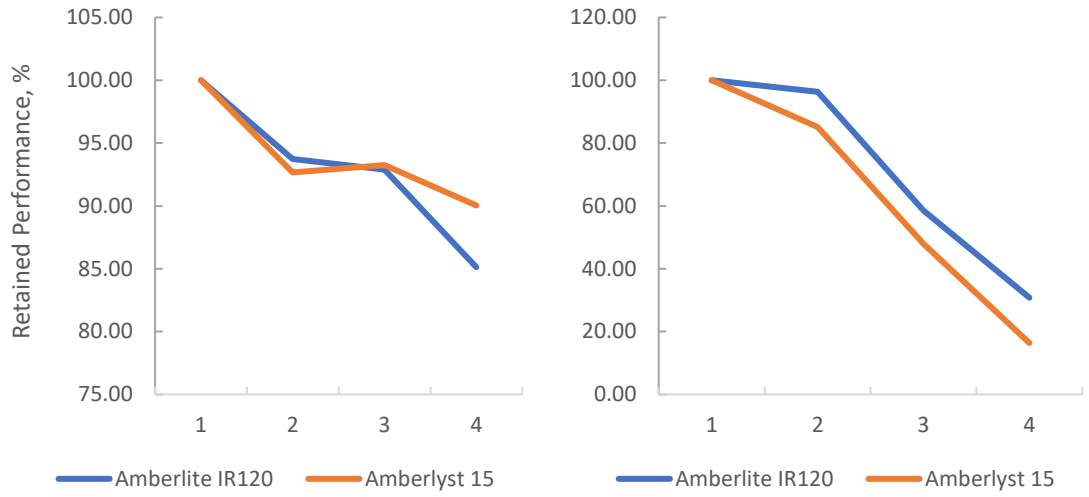

Figure 3-25- Retained performance of Amberlite IR120 and Amberlyst 15 C5 hydrolysis after 5 reutilizations on ER (left) and WS (right) liquor from organolv.

Figure 3.24 demonstrates that the behavior of IERs is similar when catalyzing both autohydrolysis and organosolv liquor. The behavioral variation between ER and WS is also observed in this case, showing that the ash concentration is a very important factor in the reuse of this type of solid acid catalysts. It is also worth mentioning that despite the presence of soluble ethanol and lignin in the medium, there was no change in the hydrolysis efficiency behavior and stability of the resins. It was expected, since, as in water, the polystyrene that constitutes the matrix of Amberlite IR120 and Amberlyst 15, is also resistant to ethanol (122).

3.5 Flow-through oligosaccharides hydrolysis

The batch results revealed a great hydrolysis potential, so the next natural step is the transition to a continuous configuration. This has several advantages over batch mode, namely: i) higher conversion, ii) higher throughput, iii) no time losses in heating, filling, cooling and emptying of the reactor, which have a negative impact on productivity, iv) superior energy efficiency, v) lower CAPEX and OPEX, and finally vi) easier controllability of the process variables (123).

It is clear that the continuous regime has a great potential for optimizing the process and it was decided to test the IER that obtained the best results in batch, Amberlyst 15, in a preliminary trial of raffinose hydrolysis with a flow-through regime. The results are presented in Figure 3-26.

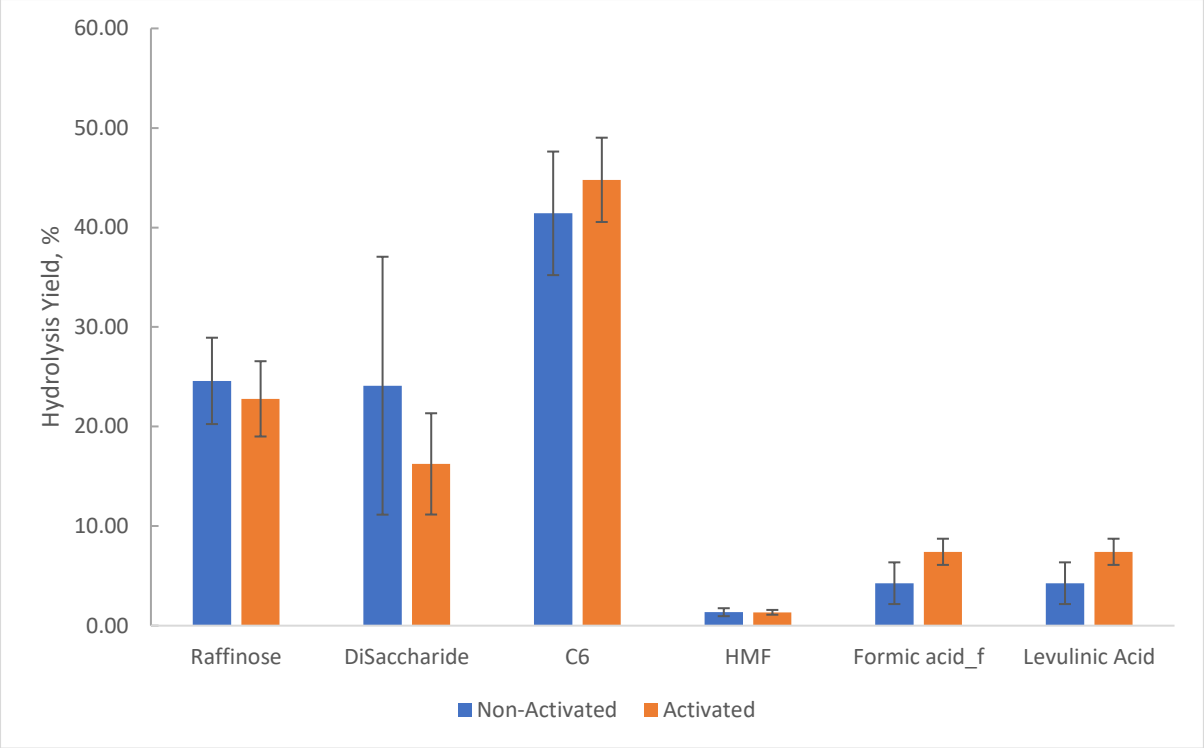

Figure 3-26- Average raffinose hydrolysis yield catalyzed by Amberlyst 15 on a flow-through regime (Dionex™ ASE™ 150) during 8 reutilizations. C6- Hexoses (Glucose, galactose, and fructose)

In the study of raffinose hydrolysis (10 g/L) in a flow-through regime, the process was performed under a temperature of 140ºC since it had previously shown good results with a batch regime. Bearing in mind that hexoses represent a total conversion of raffinose to monomers, analyzing Figure 3-26, it is possible to notice that in cycles where 2 ml of raffinose are in contact for 5 min with the catalysts, 41.4% and 44.8% of hexoses yield are obtained for Amberlyst 15 not activated and activated respectively. Partial hydrolysis, represented as a disaccharide, which corresponds to melibiose or sucrose, depending on the side where it first hydrolyzes, shows a yield of 24.1% when not activated and 16.2% when activated. In addition to the disaccharide, there were also 24.6% and 22.8% of raffinose that did not undergo any type of hydrolysis. A low percentage of HMF, formic acid, and levulinic acid (compounds resulting from the degradation of hexoses (Figure Annex 2.1) were also formed during the process.

Concluding, even though the activated Amberlyst 15 demonstrated slightly better yields, these conditions are still far from the optimal hydrolysis conditions, since 39.1% of raffinose and disaccharide, has not been converted into monomers. However, by the results achieved, it is possible to perceive the potential inherent in this flow-through regime, where at a relatively low temperature (140ºC) and a residence time of only 5 min, approximately half of the initial raffinose was fully hydrolyzed. For future work, it will be interesting to study if in shorter reaction times as in the ones possible with the flow-through regime, the autohydrolysis oligosaccharides liquors with higher ash percentages, also favor the loss of efficiency of the IERs, since during the raffinose assay there is no noticeable loss of efficiency throughout the 8 runs.

4 Conclusions and perspectives

In an integrated biorefinery framework, the recovery of hemicelluloses as monomeric sugars is vital, but current process options are restricted to the use of severe homogenous acids (such as H_2SO_4) based processes. As an alternative to these processes, this work explored the use of solid acid catalysts either directly (for raw biomass hydrolysis) or after oligosaccharide production by autohydrolysis or organosolv processes. Ion exchange resins (IERs) were the main studied catalysts that due to the presence of acid groups (such as sulfonic and Iminodiacetic acid groups) can be used as acid catalysts.

In the first stage, raffinose was used as a model oligosaccharide, The hydrolysis was carried out using four activated and non-activated IERs, morphologically different from each other, and compared to the conventional use of H_2SO_4. The resin with the best performance was Amberlyst 15 that achieved an efficiency of 90.3%. Besides these good results performing the conversion to monomers, a reuse capacity was also confirmed for at least three repetitions. Overall, it was observed that activated resins, with a matrix composed of crosslinked polystyrene divinylbenzene, with a macroporous structure and having as functional group sulfonic acid in the H^+ ionic form and higher Brönsted acid-sites shows better efficiency results. This behavior pattern was observed during all essays where these resins were tested, indicating that the use of a raffinose-based test can be a useful approach for subsequent solid acids performance screens.

In order to study the direct lignocellulosic biomass treatment to obtain monomeric hemicellulose-derived sugars, an optimization of the dilute acid hydrolysis catalyzed by H_2SO_4 was carried out. In these tests, conditions such as acid concentration and reaction time were varied, maintaining the temperature of 130ºC constant. The optimization was performed for ER and Miscanthus achieving 83.87% and 98,56% of xylan conversion into xylose, respectively.

As an alternative, the same dilute acid hydrolysis was carried out using IERs as catalysts. These solid acids, despite demonstrating a need for a higher process severity to achieve the same results as H_2SO_4, reached results of xylan yield of 93.7% for ER and 91.3% for Miscanthus. For WS, due to lower recalcitrance, total hydrolysis of the xylan in this feedstock was achieved under relatively low severity conditions (mCS of 5.06). However, due to the need for greater severity, this alternative only becomes viable if it is possible to recover and reuse the catalysts. Although efforts were directed at this, it was not yet possible to achieve an easy separation of the pretreated biomass from the catalysts.

The difficulty of recovering solid acids when direct performing dilute acid hydrolysis allied with the benefits of achieving components with better quality when performing other pretreatments before, leads to other approaches using combined processes. The objective was, in the first stage to recover hemicellulose-derived oligosaccharides (XOS) using a hydrothermal process (autohydrolysis). XOS are potential added-value products, but present a limited market volume. For this to be achieved the operational conditions leading to the highest recovery of XOS on ER were identified and it was also used for WS. High recovery of XOS at relatively mild conditions, together with lower formation of inhibitors such as HMF, furfural, and acetic acid were obtained. Organosolv process was also performed

but the conditions were optimized for lignin solubilization, resulting in lower oligosaccharides concentration when compared with autohydrolysis.

For the second stage of the two-step process, the liquid recovered from both, autohydrolysis and organosolv, were used as feedstock for a post-hydrolysis catalyzed by the solid acids and compared with the conventional quantitative acid hydrolysis. The conversion to monomers were very similar or even superior to those obtained when catalyzed by H_2SO_4.In ER autohydrolysis liquors amberlite IR120 and Amberlyst 15 not activated, achieved pentoses hydrolysis efficiencies higher than quantitative acid hydrolysis, reaching an additional 1.8% and 2.2%, respectively When activated, they demonstrate similar behavior, obtaining efficiencies of 100.6% and 99.4%. For WS, Amberlyst 15 achieved a similar behavior, reaching 98.3% non-activated and 99.1% when activated. Unlike dilute acid hydrolysis of the feedstock, in here, the recovery of the catalysts is extremely easy, being carried out by simple filtration, however, the number of possible reuses appears to be dependent on the amount of ash present in the feedstock. These combined processes allow the recovery of a liquid phase rich in XOS, in the first step and a significant monomer yield on the second step.

Based on these promising results, obtained in batch mode, and with the aim of further intensify the process, a preliminary test was performed, in a continuous regime. Again, raffinose was used as model oligosaccharide, and Amberlyst 15 was used as catalyst, either activated and not activated. Despite the tested conditions are still far from optimal, the flow-through regime showed great potential, presenting an approximately 50% hydrolysis for a residence time of only 5 min at 140ºC, without losing catalytic efficiency throughout 8 reutilizations. This is considered very promising for future applications.

Although the results presented interesting prospective, there are still issues that must be addressed in future works, to better consider a future industrial application of the developed strategy(ies).

A first step would be to direct efforts to overcome the difficulty of catalysts recovery after the direct diluted acid hydrolysis, in order to turn this process viable. A possible way forward is the further development of a magnetic catalyst that was initially explored in this work but with limited success.

Secondly, it is important to perform an economic evaluation of the two-step process, in order to ascertain the minimum catalysts reuses that are needed to turn solid acids utilization economically viable.

Finally, even though the flow-through regime showed great potential, only a few preliminary tests were carried out and it is still necessary to optimize the hydrolysis of commercial oligosaccharides with IER, and to test the autohydrolysis-derived oligosaccharides hydrolysis catalyzed by IERs.

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Supplementary material

Annex 1: Mathematical formulae

Analytical methods

The moisture content of the samples was calculated using the following expression (101):

$$\text{Moisture content (\%)} = \frac{\text{wet sample weight (g)} - \text{oven-dry sample weight (g)}}{\text{wet sample weight (g)}} \times 100$$

The ash content (Ash, %) of the samples was calculated using the following equation (102):

$$\text{Ash (\%)} = \frac{\text{Ash weight (g)}}{\text{wet sample weight (g)} \times [1 - (\text{Moisture content})/100]} \times 100$$

Concentrations of glucose, xylose, arabinose, and acetic acid in the liquors resulting from quantitative acid hydrolysis (103) of raw materials and pretreated solids were used for the calculation of glucan, xylan, arabinan, and acetyl groups content (%), respectively. The acid-insoluble residue, after correction for the ash content, was quantified as Klason lignin. During quantitative acid hydrolysis, a significant percentage of the monosaccharides is degraded, so correction factors (F) are introduced to account for the losses (124). The following expressions were used:

$$\text{Gn} = F \times \frac{100}{1025} \times \frac{162}{180} \times \frac{\text{Glc} \times P_{sol}}{A \times [1 - (\text{Moisture content})/100]}$$

$$\text{Xn} = F \times \frac{100}{1025} \times \frac{132}{150} \times \frac{\text{Xyl} \times P_{sol}}{A \times [1 - (\text{Moisture content})/100]}$$

$$\text{Arn} = F \times \frac{100}{1025} \times \frac{132}{150} \times \frac{\text{Ara} \times P_{sol}}{A \times [1 - (\text{Moisture content})/100]}$$

$$\text{Ac} = \frac{100}{1025} \times \frac{60}{61} \times \frac{\text{HAc} \times P_{sol}}{A \times [1 - (\text{Moisture content})/100]}$$

$$\text{KL} = \frac{\text{AIS-Ash}}{A \times [1 - (\text{Moisture content})/100]} \times 100$$

Where,

Gn, Xn, Arn, Ac, and KL are the concentrations of glucan, xylan, arabinan, acetyl groups and Klason lignin (g/100 g of dry solid), respectively.

Glc, Xyl, Ara, and HAc are the concentrations of glucose, xylose, arabinose, and acetic acid in hydrolysate liquors (g/L), respectively.

The terms $\frac{162}{180}$, $\frac{132}{150}$ are stoichiometric conversion factors of monomers into polysaccharides (loss of a water molecule).

The term $\frac{60}{61}$ is a stoichiometric conversion factor of acetic acid to acetyl groups (loss of a proton).

The term 1025 corresponds to the mass density of the hydrolysate (g/L), a 4% w/w solution of sulfuric acid.

F is the correction factor accounting for sugar degradation (1.04 and 1.09 for hexoses and pentoses, respectively).

W_{sol} and A are the weights of the solution and sample used in the test, respectively (g).

The moisture content respects the sample moisture content.

AIS and Ash are the weight of the acid-insoluble residue of the sample and its ash content, respectively (g).

The concentrations of gluco-oligosaccharides (GOS), xylo-oligosaccharides (XOS) and arabino-oligosaccharides (AOS) in organosolv liquors were calculated, after post-hydrolysis (106), according to the following equations:

$$\text{GOS}=(Glc_{PH} \times \text{F} \times DF - Glc_L) \times \frac{162}{180}$$

$$\text{XOS}=(Xyl_{PH} \times \text{F} \times DF - Xyl_L) \times \frac{132}{150}$$

$$\text{AOS}=(Ara_{PH} \times \text{F} \times DF - Ara_L) \times \frac{132}{150}$$

Where,

Glc_{PH}, Xyl_{PH}, Ara_{PH} are the concentrations of glucose, xylose, and arabinose in post-hydrolysis hydrolysates of organosolv liquors, expressed in g/L.

Glc_L, Xyl_L, Ara_L are the percentages of glucose, xylose, and arabinose in pretreatment liquors, expressed in g/L.

DF is the dilution factor associated with the addition of sulfuric acid 72% to the autohydrolysis and organosolv liquor, in mL/mL.

F is the correction factor accounting for sugar degradation (1.04 and 1.09 for hexoses and pentoses, respectively).

The terms $\frac{162}{180}$, $\frac{132}{150}$ are stoichiometric conversion factors of monomers into polysaccharides (loss of a water molecule).

Phenolic content/acid-soluble lignin (ASL) of hydrolysates and pretreatment liquors was calculated with the following equation (106):

$$\text{ASL } (\text{g/L}) = \frac{\text{A}}{\varepsilon \times \text{l}} \times \text{DF}$$

Where,

A is the absorbance of the liquid sample at 320 nm.

l is the length of the measuring cell (1 cm).

ε is the extinction coefficient of the acid soluble lignin, expressed in $Lg^{-1}cm^{-1}$;

DF is the dilution factor to account for the applied liquid sample dilution, in mL/mL.

Solid yield and solid component recovery in pre-treatment

Pretreatment solid yield (SY, %) was calculated from the following equation:

$$\text{SY (\%)} = \frac{\text{oven-dried mass of pretreated solids}}{\text{mass of initial feedstock} \times [1 - (\text{Moisture content})/100]} \times 100$$

The recovery of glucan, xylan, arabinan, acetyl groups, Klason lignin and ash, expressed as the percentage that remains in the solid residue after organosolv and autohydrolysis pre-treatment, was calculated according to the following equations:

$$\mathrm{Gn_R} = \frac{\mathrm{Gn} \times \mathrm{SY}}{\mathrm{Gn_F}}$$

$$\mathrm{Xn_R} = \frac{\mathrm{Xn} \times \mathrm{SY}}{\mathrm{Xn_F}}$$

$$\mathrm{Arn_R} = \frac{\mathrm{Arn} \times \mathrm{SY}}{\mathrm{Arn_F}}$$

$$Ac_{\mathrm{R}} = \frac{\mathrm{Ac} \times \mathrm{SY}}{\mathrm{Ac_F}}$$

$$\mathrm{KL_R} = \frac{\mathrm{KL} \times \mathrm{SY}}{\mathrm{KL_F}}$$

$$Ash_{\mathrm{R}} = \frac{\mathrm{Ash} \times \mathrm{SY}}{\mathrm{Gn_F}}$$

Where,

Gn_R, Xn_R, Arn_R, Ac_R, KL_R, and Ash_R are the percentages of glucan, xylan, arabinan, acetyl groups, Klason lignin and ash that remain in the residue after the organosolv pre-treatment (g/100g of the respective initial component).

SY is the pre-treatment solid yield (g of recovered solid /100 g of raw material).

Gn, Xn, Arn, Ac, KL, and Ash are the percentages of glucan, xylan, arabinan, acetyl groups, Klason lignin and ash on the pretreated solids, respectively (g/100 g of raw material), as determined by quantitative acid hydrolysis.

Gn_F, Xn_F, Arn_F, Ac_F, KL_F, and Ash_F are the percentages of glucan, xylan, arabinan, acetyl groups, Klason lignin, and ash on the feedstock, respectively (g/100 g of raw material).

Annex 2: Sugar degradation during biomass pre-treatment

The sugars released through pre-treatment of lignocellulosic materials, because of hydrolysis reactions of cellulose and hemicelluloses, can experience further reactions with subsequent formation of sugar degradation products (desirable or not). The reactions of the formation of these products are slower than the hydrolysis of polysaccharides to monomeric sugars. The reaction rate is increased by increasing temperature and/or acid concentration, for which optimization of operating conditions is mandatory to set the selectivity of biomass pre-treatment towards a specific product, being oligomeric sugars, monomers, or degradation products (125).

Glucose (hexose) can degrade into HMF for certain severities during acid pre-treatment of lignocellulosic biomass. HMF further degrades into levulinic and formic acid (1:1), according to Figure Annex 2. (126). HMF can also suffer condensation and polymerization reactions to produce humins. Humins are carbonaceous, polymeric by-products produced during acid-catalyzed dehydration of sugars by reactions of HMF, sugars, and other reaction intermediates produced during sugar dehydration and subsequent rehydration of HMF (127). Figure Annex 2. demonstrates the proposed general structure of a humin fragment by van Zandvoort et al. For more information on humins formation and characterization please see (127-129).

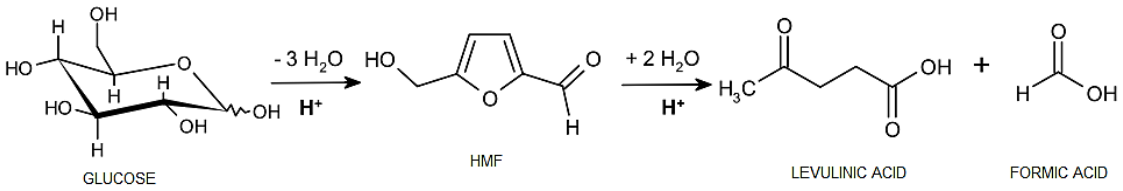

Figure Annex 2.1. Glucose degradation to HMF and the conversion of the latter into levulinic and formic acids (126)

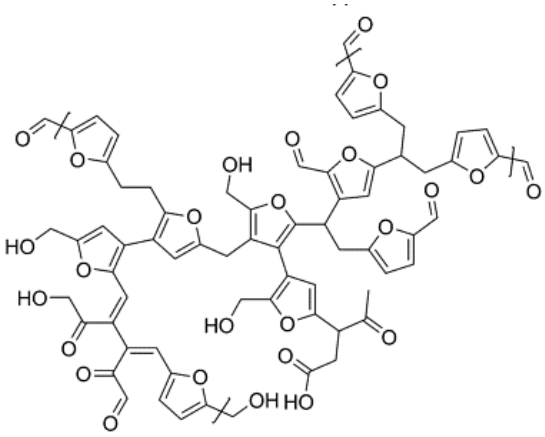

Figure Annex 2.2. Model structure of a glucose-derived humin fragment as suggested by (128)

Pentoses such as xylose, arabinose, and rhamnose are well known to yield furfural (1:1 mol ratio) in acidic media upon heating (130). With prolonged reaction time in an acid environment, furfural further degrades into other products following first-order kinetics (131). Two possible pathways are

reported for furfural degradation (Figure Annex 2.): The path of furfural resignification, in which furfural reacts with itself, and furfural condensation, in which furfural reacts with pentose-to-furfural intermediates, typically small aldehydes (125, 132, 133). The products resulting from these reactions are not completely identified given their complexity. Nevertheless, formic acid and succindialdehyde are suggested to be produced in aqueous medium by hydrolytic fission of the aldehyde group of furfural, with one mol of furfural, yielding one mol of formic acid and one mol of succindialdehyde (125). Succindialdehyde is also proposed to form humins via condensation reactions (125, 130). Those insoluble polymers can negatively precipitate into pre-treated solids during pre-treatment or into precipitated lignin, reducing product purity and the enzymatic digestibility. Higher treatment severities increase the rate of furfural degradation. Very high temperatures will inhibit the formation of insoluble polymeric products (resins) due to entropy effect (132).

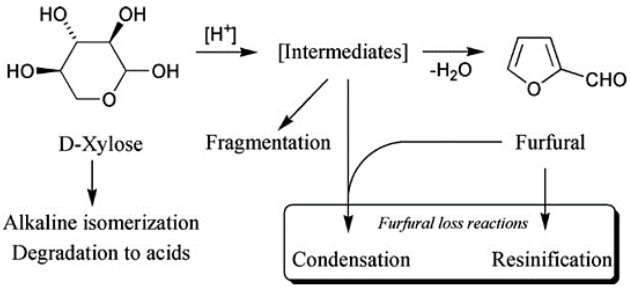

Figure Annex 2.3. Xylose and furfural degradation pathways according to (134)

Moreover, both pentoses and hexoses can degrade into several compounds, particularly aldehydes (formaldehyde, pyruvaldehyde, glyceraldehyde) and aliphatic acids (acetic, formic, levulinic) (134)

Due to the complexity and multiple reaction paths during sugar degradation that can lead to formic acid production, the attribution to a specific sugar is somewhat questionable. As a simplistic method followed in this work, the quantification of levulinic acid can be used to predict the amount of formic acid prevenient from glucose degradation, as hexose degradation yields HMF which is then degraded into formic and levulinic acid in equimolar proportions. The remaining formic acid should be attributed to degradation reactions of pentoses (xylose, arabinose) by exclusion, even though glucose can also be degraded into formic acid without ever forming HMF. However, as glucose is mostly preserved in the solid fraction during autohydrolysis and organosolv pre-treatments at mild conditions, that approximation should not be too rough of an approach. All conversions of degradation products to sugar monomers should follow a 1:1 molar ratio, as all degradation, reportedly follow that stoichiometry.

Annex 3: Ion Exchange

Basic principals

In ion-exchange, ions of a given charge (either cations or anions) in a solution are adsorbed on a solid material (the ion exchanger) and are replaced by equivalent quantities of other ions of the same charge released by the solid. The ion exchanger may be a salt, acid, or base in a solid form that is insoluble in water but hydrated.

Ion exchange forms the basis of many chemical processes which can be divided into three main categories: substitution, separation, and removal of ions.

1) Substitution. A valuable ion (e.g., copper) can be recovered from solution and replaced by a worthless one. Similarly, a toxic ion (e.g., cyanide) can be removed from the solution and replaced by a nontoxic ion.

2) Separation. A solution containing several different ions passes through a column containing beads of an ion-exchange resin. The ions are separated and emerge in order of their increasing affinity for the resin.

3) Removal. By using a combination of a cation resin (in the H^+ form) and an anion resin (in the OH− form), all ions are removed and replaced by water (H^+OH^-). The solution is thus demineralized (112).

Ion Exchange Resins

IER are small beads, with a diameter of about 0.6 mm. These beads are porous and contain water inside, measured as “humidity” or “moisture content”. The structure of the resin is a polymer on which a fixed ion has been permanently attached. This ion cannot be removed or displaced making it part of the structure. To preserve the electrical neutrality of the resin, each fixed ion must be neutralized with a counterion. This counterion is mobile and can get in and out of the resin bead. Figure Annex 3.1 -Examples of cation and anion exchange resin beads structureshows a schematic cation exchange resin bead. The dark lines represent the polymeric skeleton of the resin bead. This structure is porous and contains humidity. The fixed ions of this cation exchange resin are sulphonates (SO_3^-) that are attached to the skeleton. In this picture, the mobile ions are sodium (Na+) cations.

The anion resin bead has a very similar skeleton. The functional groups are quaternary ammonium cations, shown in the figure as N^+R_3. Each ion going into the bead must be replaced by an ion getting out of the bead, to preserve electrical neutrality. This is what is called ion exchange.

Since the biomass hydrolysis is performed in acid media, the research work will be focused on acidic cation exchange resins.

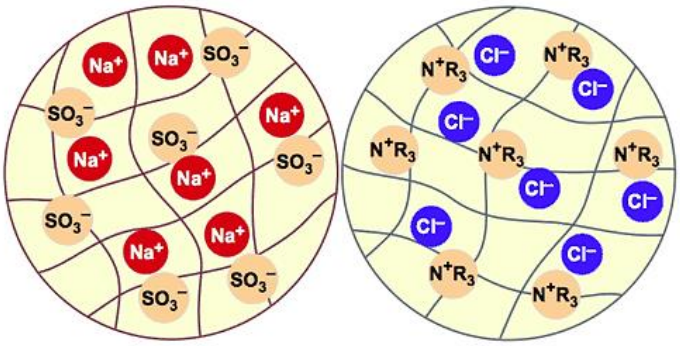

Figure Annex 3.1 -Examples of cation and anion exchange resin beads structure

Structures

An ion exchanger structure is composed of the polymer matrix and the functional groups that interact with the ions.

Polymer Matrices

Polystyrene Matrix- (Polystyrene and Styrene Copolymers). The polymerization of styrene under the influence of a catalyst (usually an organic peroxide) produces linear polystyrene. Linear polystyrene is a clear moldable plastic that is soluble in certain solvents (e.g., styrene or toluene) and has a well-defined softening point. To produce a completely insoluble polymer, a proportion of divinylbenzene is mixed with styrene, resulting in cross-linked polymer represented in

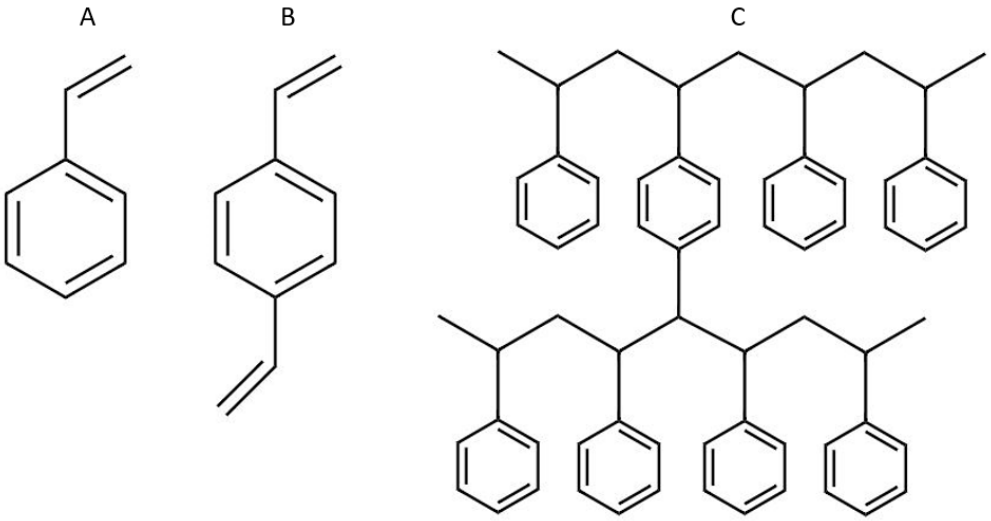

Figure Annex 3.2 - A-Styrene B-Divinylbenzene C-Polystyrene cross-linked with Divinylbenzene

The polymerization of polystyrene cross-linked with divinylbenzene, in the production of ion-exchange resins, generally occurs in suspension. Monomer droplets are formed in water and, upon completion of the polymerization process, they become hard spherical beads.

Gel and macroporous resin structure

In the polymerization process described above, the cross-linker is evenly distributed throughout the matrix. The voids between the chains of polystyrene are called pores. They are small and their size is relatively constant. The matrix has a pseudo-crystalline structure, similar to glass, and as a result, the finished IER beads are transparent. In the picture below, the polystyrene cross-linked with DVB, called the gel-type matrix, is shown.

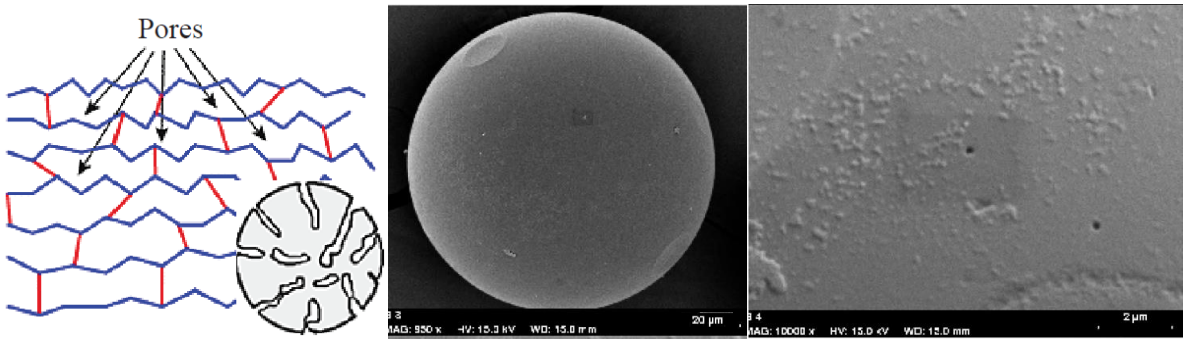

Figure Annex 3.3 - Example of a gel-type matrix. Polystyrene chains are shown in blue without the aromatic chemical details, and the "bridges" formed by DVB are shown in red. SEM images of gel-type matrix IER. Adapted from (135, 136)

There is a limit to the quantity of DVB that can be used in gel-type resins. An increase in the degree of cross-linking (i.e., the weight percentage of DVB related to the total amount of monomer before polymerization) produces harder, less elastic resins. Resins with higher degrees of cross-linking show more resistance to oxidizing conditions that tend to break the crosslink of the polymer. However, above 10-12% DVB, the structure becomes too hard and dense resulting in greater activation difficulty (i.e., chemical transformation of the inert copolymer into an ion-exchange resin) because access to the interior of the bead is hindered by the high density of the matrix. Furthermore, with less elasticity of the structure, osmotic stress cannot be absorbed causing the beads to shatter. Also, the rate of exchange increases in proportion to the mobility of the ions inside the exchanger bead, which means, if the structure is too dense, ionic motion is slowed down and consequently reduces the operating capacity of the resin. (i.e., for sulfonic resins with a gel matrix, the maximum operating capacity is reached with approximately 8% DVB). Crosslinking reduces the retention of water in IERs and the volume occupied by this water is a measure of the resin's porosity. However, cross-linking is not uniform because the DVB–DVB reaction is faster than that between DVB and styrene. Polymerization begins to happen around the catalyst molecules, and polymer growth is faster at sites rich in DVB than at those rich in styrene. Material with an average of 8% DVB may contain local microscopic regions with more than 20% DVB, whereas other regions may have less than 4%.

To overcome the limitation of the DVB quantity problem, macroporous resins have been invented in the 1960s. The idea is to create artificial porosity in the tri-dimensional matrix. Macroporous resins are made by mixing the monomers with a porogen (e.g., heptane, saturated fatty acids, C_4 –C_{10} alcohols or polyalcohols, or low molecular mass linear polystyrene) which expands the resin. The substance does not itself polymerize and, thus, although it acts as a solvent for the monomers, it causes the polymer to precipitate from the liquid. They form opaque round particles and have large surface areas (137). Once the polymerization reaction is finished, the porogen is washed out and leaves voids in the polymer

structure. Macroporous resins have a higher degree of cross-linking than gel resins to strengthen the matrix and compensate for voids left by the added solvent. The porosity and mechanical strength of the resin can be modified by varying the degree of cross-linking or the amount of solvent added. Therefore, various macroporous resins are available, with different moisture-holding capacities and internal structures. The pore diameter is circa 100 nm in a macroporous resin and circa 1 nm in a gel resin. The macropores form a network of channels filled with free water, and large molecules can move freely in the resin into the center of a bead. Once inside the resin, ions generally have a much shorter distance to travel before they encounter an active group, circa 100 nm in macroporous resins, and up to 500 µm in gel resins and consequently, the exchange is faster in a macroporous resin. Macroporous resins are highly resistant to physical stress and generally withstand osmotic shock very well. (112)

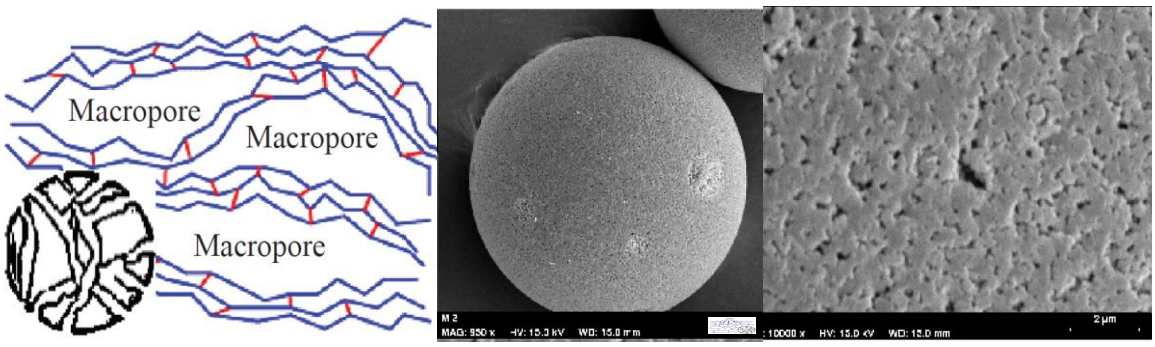

Figure Annex 3.4 -Example of macroporous type matrix. Polystyrene chains are shown in blue without the aromatic chemical details, and the "bridges" formed by DVB are shown in red. SEM images of macroporous type matrix IER. Adapted from (135, 136)

Perfluorinated Resin Sulfonic Acid (Nafion-H®)

The perfluorinated matrix is a peculiar kind of backbone of Nafion, one of the IERs tested in this research work. It was discovered in the late 1960s by Walther Grot at DuPont. The membrane consists of a perfluorinated backbone (for chemical stability) and pendant chains terminated by sulfonic groups, SO3H- (for ionic conductivity) (138). Therefore, it combines two extremes. The perfluorinated sulfonic acid side chains are strongly hydrophilic, while the perfluorinated backbones are strongly hydrophobic. The conductivity of Nafion comes from the protons of the sulfonic acid groups. The hydrated $-SO3^{-}$ side chain end groups and the absorbed water provide the media for the transport of protons. The excellent chemical resistance of Nafion comes from the PTFE like backbone. The Carbon-Fluorine bond energy is one of the highest known values, 480 kJ/mol. The combination of the proton conductivity and the chemical resistance makes Nafion widely useful for electrochemical processes.

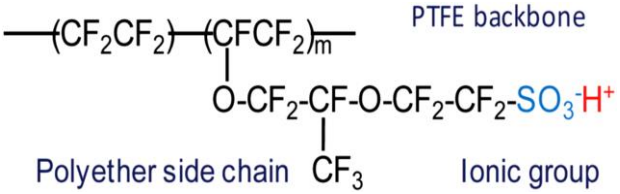

Figure Annex 3.5 - The chemical structure of Nafion polymer (139)

Nafion is synthesized by the copolymerization of tetrafluoroethylene (the monomer of Teflon) and a derivative of a perfluoro (alkyl vinyl ether) with sulfonyl acid fluoride.

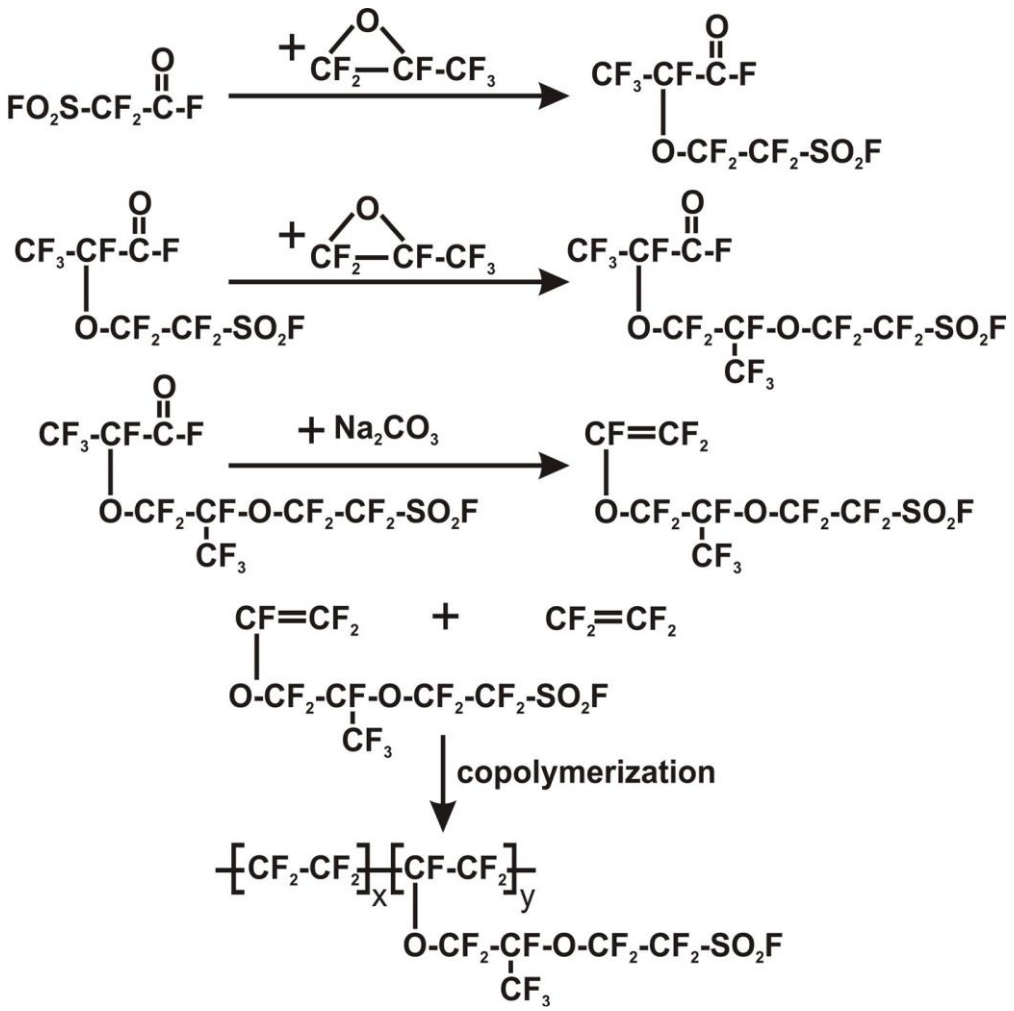

Figure Annex 3.6 - Synthesis of DuPont's Nafion. (Adapted from (140))

Other matrices

Polyacrylic Matrix. Matrices for ion exchangers can also be obtained by polymerizing an acrylate, a methacrylate, or acrylonitrile. These matrices can, also, be cross-linked with divinylbenzene.

Phenolic resins which show interesting adsorption properties.

Polyalkylamine resins, obtained from polyamines by condensation with epichlorohydrin, which gives an anion exchanger directly in a single step.

Functional Groups

This section can be divided into cation and anion exchange resins, however, only cation exchange resins were addressed in this work.

Cation-Exchange Resins

Cation-exchange resins in current use can be separated into two classes according to their active groups:

1) Strongly acidic (sulfonic groups)

2) Weakly acidic (carboxylic groups)

The sulphonic active group ($-SO_3^-$) and the carboxylic ($-COO^-$) are the most common strong acid and weak acid active groups, respectively. The dissociation constant for the sulphonic group ($-SO_3-H^+$) is

extremely high even at low pH's resulting in a strong acid resin, while in the case of the carboxylic group (-COO-H^+) the hydrogen ion is held far more strongly giving a far lower dissociation constant and hence a weak acid resin is the result. Higher pH's are required for adsorption so as to allow for deprotonation of the carboxylic acid before it becomes available for the adsorption of alternate cationic species (141).

Strongly Acidic Cation-Exchange Resins

Chemically inert polystyrene beads are treated with concentrated sulfuric or chlorosulfonic acid to give cross-linked polystyrene 3-sulfonic acid. After sulphonation, the resin is washed to remove excess sulphuric acid. This hydration step is a delicate operation, as it causes the resin beads to swell (functional groups are hydrated and thus grow). The corresponding osmotic force is considerable and can result in breaking the beads to pieces if it is not done cautiously. This reaction produces the resin in the hydrogen form. This material is the most widely used cation exchange resin and is strongly acidic. Amberlite IR 120 and amberlyst 15, solid acids studied during this research, are examples of these kinds of exchange resins.

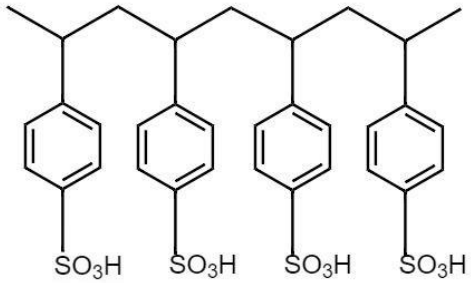

Figure Annex 3.7 -Polystyrene sulphonate

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Weakly Acidic Cation-Exchange Resins

The weakly acidic resins are almost always obtained by hydrolysis of polymethylacrylate or polyacrylonitrile to give a poly (acrylic acid) matrix. The WAC resin product obtained is a weak acid, only partially ionized in a neutral environment. Its acidity is similar to that of acetic acid. Due to the aliphatic (not aromatic) light-weight structure of the matrix, WAC resins have a higher density of active groups than resins based on polystyrene. This results in a high total ion exchange capacity.

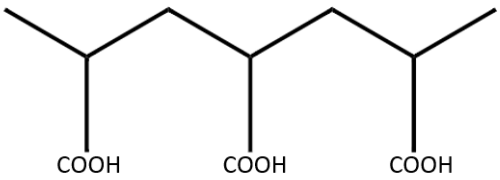


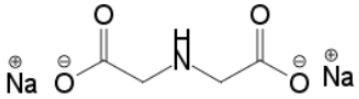
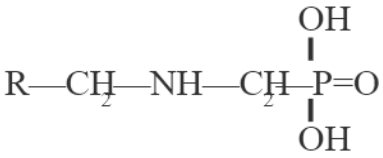
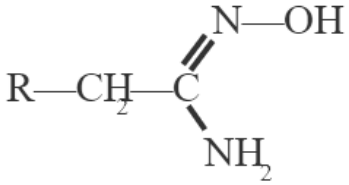
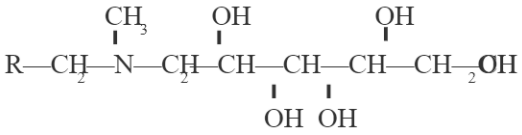
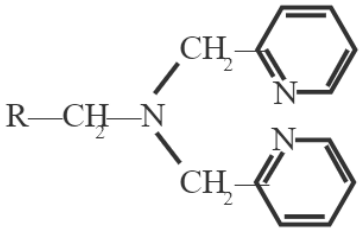
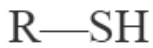
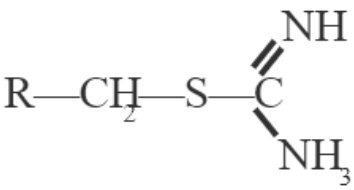
Figure Annex 3.8 - Poly(acrylic acid) matrix

Other Types of Ion-Exchange Resins

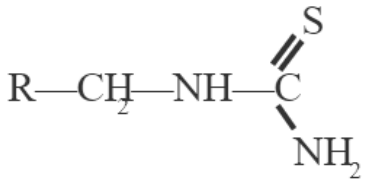
By using polymerization and activation methods analogous to those described above and the ones used in anion exchange resins, a wide variety of functional groups can be grafted onto a given polymer. Some of these groups can be used for selective uptake of ions, principally metals. The iminodiacetic, aminophosphonic, and amidoxime groups form metal complexes whose stability depends mainly on the pH of the solution. Selective adsorption of certain metals can consequently be achieved by varying the pH. These types of material are known as chelating or complexing resins. The chelating resins make complexes only with multivalent metals, which are very stable. Therefore, these resins have high selectivity and can, preferentially, remove metals from solution.

In the table below, some of these selective functional groups are presented.

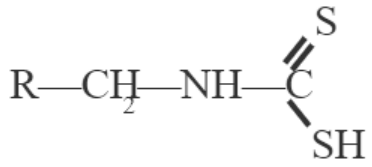
Table Annex 3.1-Examples of selective functional groups structures

Iminodiacetic	
Aminophosphonic	
Amidoxime	
N-methyl glucamine	
Bis-picolylamine	
Thiol	
Thiouronium	

Thiourea



Dithiocarbamat



One of these selective functional groups was tested in biomass hydrolysis during this work. Amberlite IRC748 is an iminodiacetic acid chelating cation exchange resin with high selectivity for calcium, magnesium, and strontium in chloralkali brines. This resin also exhibits high selectivity for heavy metal cations over alkali metal ions found in various processes and waste streams. Selectivity is achieved by the iminodiacetic acid functionality, chemically bound to a macroreticular resin matrix. Because of the high preference of Amberlite IRC748 for metals and excellent kinetic performance, this resin can remove metals from solutions even in the presence of high concentrations of sodium or calcium salts, with very low metal leakage. The macroreticular structure of Amberlite IRC748 is highly resistant to osmotic shock and has excellent physical stability.

Properties

Exchange Capacity

The capacity of the resin is generally measured in ‘equivalents per volume’ as there is considered to be a direct link between active sites and capacity, the copolymer matrix of the resin being assumed homogeneous concerning active site concentration. The term ‘equivalents’ is used and could be paralleled to the term ‘Normal’ in standard wet chemistry calculations. Essentially, equivalents per volume refer to the molar quantity of monovalent ions adsorbed per unit volume, which implies that the number of divalent ions that the same resin could adsorb would be half as much i.e. double the number of active sites would now be required to accommodate the same amount of divalent atoms/molecules.

The volume of the resin can be linked to the density of the fixed ionic groups, with the number of ionic groups per bead being the only constant. However, any swelling of the resin, a common phenomenon especially with strong acid resin in the H+ form, will alter this specific capacity. The measurement of the actual volume of the resin also has its difficulties and is normally achieved and reported as Free-Wet-Settled-Volume (FWSV) i.e. the resin is allowed to settle in a measuring flask and the volume recorded. The accurate measurement of this could be adversely impacted by the time left to settle, the temperature of the liquid (generally aqueous), and the pH of the solution.

Resin capacity and its measurement in equivalents/volume is in itself an imprecise element as it can vary with changes in electro-negativity of the absorbing ion and degree of conversion i.e. it links to equilibrium concentration and this is especially true for strong acid and base resins. The extent to which the continuous solution-phase associated with the resin can be considered an infinite solution is also a factor that plays a role in the measurement, as it is only in a truly infinite solution i.e. no desorbing ion

present, that maximum capacity can be determined. As most IER applications are in either a fixed or fluidized bed arrangement through which the continuous medium passes, it is reasonable to assume that an infinite solution system exists in practice. However, it may not be desirable for the resin to be operated beyond its breakthrough point as this may mean loss of valuable ion being recovered or the discharge to the product of undesirable ions in solution. Ultimately useful capacity is invariably less than the actual capacity.

Resin capacity can also be described as equivalents per dry weight (milliMolequivalents/gram) a measurement that requires the destruction of the resin for analytical purposes. Arguably, the achieved measurement has a strong link to the mass of polymer present and it is necessary to make sure that there are no losses during the destruction process, particularly if it is via thermal desiccation. However, linking this value to the ion capacity of hydrated fresh resin, where only the difficult-to-measure volume of the resin bed is known, makes it a tenuous measurement at best. In a finite system where the quantity of resin and the associated aqueous solution is constant, the equilibrium thus established between the desorbing and adsorbing ion would likely be disturbed if a quantity of resin were removed to determine the MilliMole-quivalents/gram measurement, making this measurement inaccurate under these circumstances (141).

Another manner of describing the resin capacity is the method used in this work, that stood out for the relationship between its effectiveness and the reduced number of process difficulties, where the Brönsted acid-sites were determined by titration and quantified in mmol g^{-1} (107).

Stability

The chemical stability of IERs can be put at the risk since the cross-linking present in the matrix can be damaged if exposed to highly oxidizing conditions. The capacity of a sulfonated polystyrene cation-exchange resin with 8% DVB cross-linking is dependant on the temperature. At ambient temperature, these resins can withstand 0.2 mg/kg of chlorine for several years but to be able to resist at 120ºC the environment must be free of oxidants. This oxidation process provokes the cross-linking breakdown, releases sulfonated organic compounds and causes the resin to swell until it softens, resulting in degradation. The macroporous resins should be used when oxidizing agents are present because of their highly cross-linked percentage a consequently greater resistance to oxidation.

The cation exchange resins have greater thermal stability than anion exchange resin, degrading 1.6-7 times slower. (142) Elevated temperatures can influence the conductivity, affecting the value of ion-exchange capacity and functionality of the IER (143). Degradation by thermal loading in the case of cation-exchange resins with functional sulphonic groups starts by the dehydration of sulphonic acid and then decomposition into sulfur dioxide(144)

Analyzing the mechanical stability, polystyrene and polyacrylic resins are perfect spheres made by suspension polymerization and almost endure no damage when used in continuous moving-bed ion-exchange plants. Although, mechanical strength can differ considerably between products, and resin beads with many internal cracks under the microscope are more likely to break under mechanical stress than crack-free products. Gel-type cation materials are generally stronger than anion resins and can withstand a greater level of compression. Acrylic resins have better elastic capacity than polystyrene materials and can usually withstand any mechanical stress. Macroporous resins are considered the

strongest and are extendedly used for the most severe stress conditions. Resins with the highest degree of cross-linking have the less elastic capacity (gel resins with >8%DVB and macroporous resins with>15% DVB), and when they break, they explode into a lot of fragments, while other resins break into two or three usable pieces.

During ion exchange, the configuration around each active group shifts. The adsorbed ion usually has a different size and a different hydration layer than the displaced ion, therefore the resin beads may swell or contract during the reaction. Throughout these volume changes the resin is subjected to stresses known as osmotic forces. These forces can reach local pressures of several thousand kilopascals greatly exceeding the purely mechanical stress. Resins for industrial use must be able to withstand hundreds of cycles of exhaustion and regeneration. Nature and, consequently, the strength of osmotic shock that the resins withstand, change according to the ionic species in solution and their concentration. Resins can achieve higher limits of mechanical and osmotic stress with a matrix that is not only strong enough to withstand physical shock (attrition) but also flexible and porous enough to deform without breaking under the effect of osmotic shock. Some macroporous resins combine both qualities.

Particle Size

Particle size is a compromise between the speed of the exchange reaction (better with smaller beads) and high flow rates (thick particles to minimize the head loss), to obtain the desired results in industrial use. The polymerization technology, the suspension medium, and the monomer concentration define the size of the polymer droplets formed during polymerization, and therefore the size of the resin beads. Traditional polymerizations, which produce beads with a range of particle sizes rather than a uniform size, are carried out in batches in a stirred reactor. The population of a resin sample (i.e., the number of beads classified according to bead diameter) has an approximately Gaussian distribution.

For Gaussian distribution, the particle-size distribution is defined by:

1. The mean diameter. (Corresponding to the mesh size of a sieve allowing 50% of the beads to pass).
2. The uniformity coefficient. (Given by the ratio between the orifice of a sieve allowing 60% of the beads to pass and that of a sieve allowing 10% to pass).

The closer the uniformity coefficient is to unity, the narrower is the Gaussian curve and the smaller is the range of particle size. Resins produced with the traditional stirred reactor process usually have a uniformity coefficient of 1.5 to 1.9.

Different polymerization new technologies allow several producers to offer ion-exchange resins with a very uniform particle size distribution. (i.e., in one of these techniques, the monomers are injected into the suspension medium through a plate perforated with thousands of very small holes. Droplets of the monomer mixture with an almost identical volume are expelled. The uniformity coefficient of resins produced in this way is always less than 1.2.

Moisture Content

The water retention capacity (water molecules that surround both the mobile and fixed ions located in the interior of the resin beads) manage the kinetics, exchange capacity, and mechanical strength of ion-exchange resins. The moisture content or moisture-holding capacity (MHC) is defined as

$$MHC = \frac{(P_{Hydr} - P_{Dry})}{P_{Hydr}}$$

where PHydr is the weight of the hydrated resin sample and PDry the weight of the same sample after drying. The MHC of an ion-exchange resin is an inverse function of the degree of cross-linking. In macroporous, resins, where the porosity or degree of cross-linking in the polymer is artificially increased, the behavior is different.

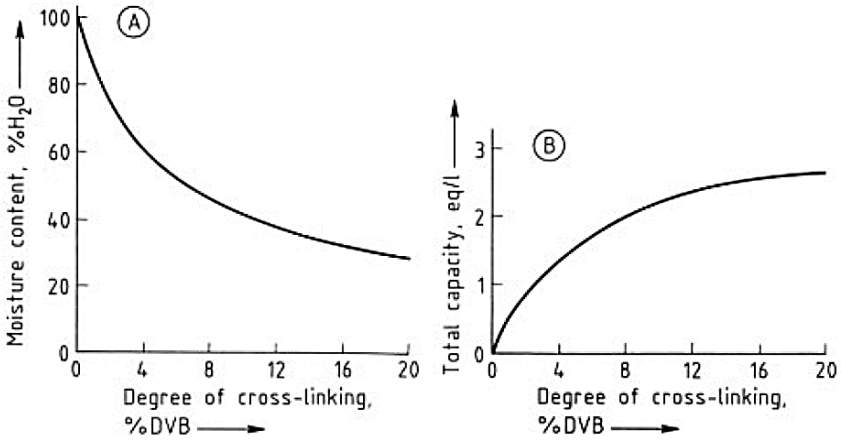


Figure Annex 3.9 -Variation of moisture content (A) and total capacity (B) with the degree of cross-linking in a gel type sulfonated polystyrene resin in sodium form

Annex 4: List of publications

Full articles:

1. Pinto F., Duarte L.C., Carvalheiro F., Paradela F., Costa P., Marques J., Andre R., Marques P., Costa D., Sampaio B. 2019. "Production of liquid compounds by co-Pyrolysis of different pre-treated biomasses mixed with plastic wastes". *Chemical engineering transactions*. https://doi.org/10.3303/CET1976233

Conference proceedings

1. Costa D, Carvalheiro F, Duarte LC, Gírio F (2019) Efficient hydrolysis of hemicellulosic oligosaccharides using solid acids. In: Carvalho MdG, Scarlat N, GrassI A, Helm P (eds) *Papers of the 27th European biomass conference. Setting the course for a biobased economy.* ETA-Florence Renewable Energies, Florence, Italy, pp 1317-1319. https://doi.org/10.5071/27thEUBCE2019-3CV.5.20

Conference communications

1. Carvalheiro, F., R. M. Lukasik, L. C. Duarte, L. B. Roseiro, B. Ribeiro, S. Marques, J. R. Bernardo, V. Van-Dunem, F. Pires, D. Costa, L. Sanfins and F. Gírio (2020). Desenvolvimento de processos de pré-tratamento da biomassa para a separação eficiente das correntes de lenhina e de açúcares. CIES2020. Lisbon, Portugal.

2. Costa D, Carvalheiro F, Duarte LC, Gírio F (2020) Efficient hydrolysis of lignocellulosic feedstocks using solid acids. Submitted to the *28th European biomass conference & exhibition, Marseille, France,* but removed due to the COVID-19 pandemic crisis

3. Costa, D; Vicente, D; Gomes, M; Prytysyuk, Y; Sampaio, B; Van-Dúnem, V; Sanfins, L. 2020. The impact of pretreatment process on the biomass upgrade by biochemical and thermochemical routes. Submitted *to 28th European biomass conference and exhibition - EUBCE 2020* , but removed due to the COVID-19 pandemic crisis

4. Costa D, Carvalheiro F, Duarte LC, Gírio F (2019) Efficient hydrolysis of hemicellulosic oligosaccharides using solid acids. *27th European biomass conference. Setting the course for a biobased economy. Lisboa, Portugal*

5. Vilcocq L, Duarte LC, Costa D, Sampaio B, Crepet A (2019) Autohydrolysis and catalytic hydrolysis processes for rare sugars production from agricultural waste. Paper presented at the *5th International congress on catalysis for biorefineries (CatBior V), Turku, Finland.*